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COMPARATIVE BIOCHEMISTRY

VOLUME III: CONSTITUENTS OF LIFE—PART A

COMPARATIVE BIOCHEMISTRY

A Comprehensive Treatise

Volume I: Sources of Free Energy
Volume II: Free Energy and Biological Function
Volume III: Constituents of Life—Part A
Volume IV: Constituents of Life—Part B
Volume V: Constituents of Life—Part C
Cells and Organisms—Part A
Volume VI: Cells and Organisms—Part B

COMPARATIVE BIOCHEMISTRY

A Comprehensive Treatise

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Volume III

CONSTITUENTS OF LIFE—PART A

1962

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Preface

In order to provide a systematic comparison of the biochemical phenomena of life throughout the phylogenetic scale, *Comparative Biochemistry* has been organized as follows: Volumes I and II are primarily concerned with the biological transformations of energy, Volumes III and IV with the biological transformations of matter, and Volumes V and VI with the properties of the organized systems occurring in living organisms.

Thus, the present volumes, III and IV, are devoted to the principal classes of constituents of cells and organisms, their distribution, and the comparative enzymology of their biogenesis and metabolism. The comparison of *structure and distribution*, on the one hand, and *metabolism*, on the other, require different types of specialized knowledge; we have asked two different authors, when necessary, to describe these different aspects of the composition of living organisms.

In the main, organisms are made up of fatty acids and lipids, mono- and polysaccharides, amino acids and proteins, nucleotides and nucleic acids, and water. This is an aspect of biochemical unity to which, on earth at least, there appears to be no exception. It is logical to arrange the chapters in these volumes to emphasize this unity. There are also numbers of other structural classes of metabolic components which occur only in portions of the phylogenetic scale; these less usual components illustrate the diversity of life.

The editors, although they have preferred to delay publication of individual volumes rather than have chapters appear out of their organized context, have been confronted with serious difficulties arising from the necessity of translating manuscripts written in languages other than English, and of insuring the publication of texts already received without too long a delay. They have therefore decided not to postpone the printing of chapters already on hand. This somewhat upsets the original plan of organization which, nevertheless, continues to underlie the treatise as a whole.

Among the chapters of Volume III of *Comparative Biochemistry* is printed the last publication of Professor W. Bergmann of Yale University. The chapter he has written is the result of many years of study, and will remain a testimony to his high competence in this field, as well as a sign of his devotion to comparative biochemistry, of which he was one of the undisputed pioneers.

The publishers have continued to provide us with prompt and reliable technical assistance, and we are grateful to them.

M. Florkin
Liège, Belgium

H. S. Mason
Portland, Oregon

December, 1961

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[*] Most of the names refer to phyla, except in a few cases where some of the smaller taxonomic groups are shown. Capitalized names written across lines are groups including all forms above the name.

NOTE: Charts I, II, and III were prepared by Helen A. Stafford, Reed College, Portland, Oregon. For further information see "A Guide to the Nomenclature and Classification of Organisms," by Dr. Stafford, in Vol. I of this treatise.

CHART I
HYPOTHETICAL PHYLOGENETIC RELATIONSHIPS BETWEEN EXTANT MAJOR GROUPS OF ORGANISMS*

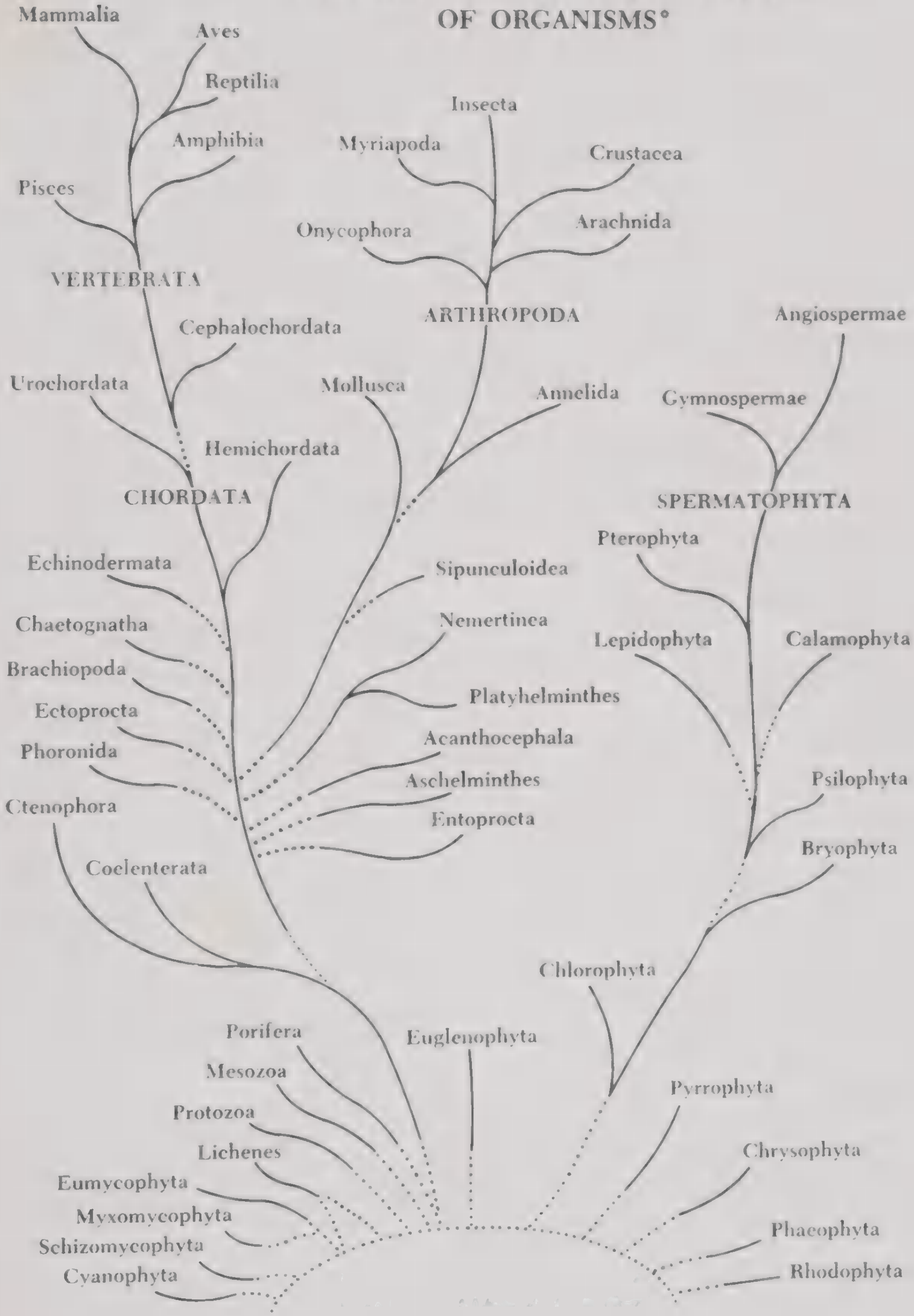

CHART II: ANIMAL KINGDOM

Divisions	Estimated Number of Species[d]	Taxonomic Classifications			
Protozoa (acellular animals)	15,000				
Mesozoa	–				
Porifera (sponges)	5,000				
Coelenterata (coelenterates)	10,000				Radiata
Ctenophora (comb jellies)	100				
Platyhelminthes (flat worms)	6,000	Acoelomates		Protostomia	Bilateria
Nemertinea (nemertine worms)	500				
Aschelminthes[a]	7,000	Pseudocoelomates			
Acanthocephala[a]					
Entoprocta[b]	3,000				
Ectoprocta[b] (moss animals)		Eucoelomates	Schizocoela		
Phoronida	15				
Brachiopoda (lamp shells)	120				
Mollusca (mollusks)	70,000				
Sipunculoidea	–				
Annelida[c] (segmented worms)	6,500				
Arthropoda (arthropods)	750,000				
Chaetognatha (arrow worms)	30		Enterocoela	Deuterostomia	
Echinodermata (echinoderms)	5,000				
Hemichordata	60,000				
Chordata (including vertebrates)					

---

[a] Includes Rotifera, Gastrotricha, Kinorhyncha, Nematoda, Nematomorpha, Priapuloidea. Formerly called Nemathelminthes.

[b] Formerly in Bryozoa.

[c] Includes Echiuroidea.

[d] Taken from "Handbook of Biological Data" (*4*), p. 533.

CHART III: PLANT KINGDOM

Divisions	Estimated Number of Species[d]	Major Synonymous Terms		
Euglenophyta (euglenoids)	340	Algae	Thallophyta	Cryptogamia
Chlorophyta (green algae)	5,700			
Pyrrophyta (cryptomonads, dinoflagellates)	1,000			
Chrysophyta (yellow green algae, diatoms)	5,700			
Phaeophyta (brown algae)	900			
Rhodophyta (red algae)	2,500			
Cyanophyta[a] (blue-green algae)	1,400			
Schizomycophyta[a] (bacteria)	1,300[e]	Fungi		
Myxomycophyta (slime molds)	430			
Eumycophyta (true fungi)	74,000			
Lichenes (lichens)	15,500			
Bryophyta (mosses and liverworts)	23,800		Bryophyta	
Psilophyta[b] (whisk ferns)	3	Psilopsida	Tracheophyta	
Calamophyta[b] (horsetails)	30	Sphenopsida		
Lepidophyta[b] (lycopods)	1,300	Lycopsida		
Pterophyta[b, c] (ferns)	10,000	Pteropsida		
Spermatophyta (seed plants)	201,000			Phanerogamia

[a] Sometimes grouped as Schizophyta.

[b] Formerly classed as Pteridophyta.

[c] Formerly classed as Filicineae in Pteropsida.

[d] Taken from "Handbook of Biological Data" (*4*), p. 533.

[e] There is much disagreement concerning designation of species here.

CHAPTER 1

The Comparative Aspects of Fatty Acid Occurrence and Distribution

F. B. SHORLAND

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I. Introduction

In living tissues fatty acids are present in a variety of compounds. These include mainly triglycerides, or true fats, and to a lesser extent phospholipids, waxes, and sterol esters. Free fatty acids do not normally occur in living tissues in more than trace amounts. The amounts of fatty substances, or lipids, range from less than 1% in the bodies of certain fish to more than 50% in some seeds and in well-fattened farm animals. A few microorganisms, especially yeasts, when suitably cultured produce much fat.

In animals the amount of fat or triglyceride is affected markedly by the plane of nutrition whereas the amounts of cholesterol and of phospholipid remain approximately constant (*1*). To distinguish between these two groups of lipids, Terroine (*2*) suggested the terms "élément variable" and "élément constant."

A wide range of fatty acids is found in nature. The depot fats synthesized by animals consist chiefly of palmitic (25–30%), oleic, and stearic acids. In addition, the dietary linoleic and linolenic acids of plant origin are elongated and desaturated in the animal. In this way are formed the characteristic polyenoic C_{20} and C_{22} unsaturated acids not found in the ingested vegetable fats. The depot fats also include considerable amounts of unmodified dietary fatty acids.

In ruminant fats the presence of positional and *trans* isomers of the naturally occurring unsaturated acids is another deviation from the simple mixtures of fatty acids characteristic of animal fats. Also in ruminant fats occur minor or trace amounts of *n*-odd, iso, and (+)-anteiso acids. These originate possibly from the lower homologs arising from breakdown of carbohydrates and proteins in the rumen.

The fatty excretions of the skin, including wool grease, hair lipids, and the preen gland lipids of birds, offer a somewhat more complex pattern of fatty acid distribution. In some skin lipids occur abundant amounts of branched-chain fatty acids as well as the *n*-odd series of saturated and unsaturated fatty acids. In the lipids of microorganisms and higher plants, the fatty acid mixtures may consist largely of oleic and linoleic acids, sometimes with linolenic acid as the main unsaturated component together with palmitic acid, which usually forms less than 10% of the total fatty acids. Frequently, however, unusual fatty acids characteristic of the species also occur as major constituents. Thus, in tubercle bacilli higher molecular weight branched-chain fatty acids predominate. In the seed fats of the higher plants there are many examples of the occurrence of specific fatty acids. In the Umbelliferae, petroselinic (octadec-*cis*-6-enoic) acid largely replaces oleic acid, while in some species, notably in the Euphorbiaceae, the conjugated C_{18} trienoic-α-eleostearic (octadeca-*cis*-9,*trans*-11,*trans*-13-trienoic) acid, is prominent. As examples of the diversity of the plant fatty acids, the occurrence may be mentioned, in a limited number of species, of hydroxy-substituted and keto-substituted unsaturated acids, unsaturated acids with acetylenic linkages, and acids with cyclopentene and cyclopropane rings, as well as epoxy unsaturated acids described in the next section.

In aquatic life there is no marked distinction between the fats of plants and animals. Throughout the whole of aquatic life the pattern of fatty acid composition is uniform, apart from minor differences between marine and freshwater species. The distinctive nature of the fat of aquatic species, which includes low amounts (15–20%) of saturated acids, mainly palmitic acid, together with a wide range (C_{14}–C_{22}) of unsaturated acids, makes it desirable to discuss the fats of aquatic species separately from those of terrestrial plants and animals.

Our knowledge of the composition of naturally occurring fats is based on the study of relatively few species and has been confined more or less to seed fats and animal depot fats, including fish oils, which are of actual or potential industrial importance. Much information has been obtained on the tissues and organs of rats because of the widespread use of this animal for experimental purposes, but little is known about the fatty substances of the lower forms of plant life. In the higher plants, the lipids in parts other than the seeds, including leaves, roots, and flowers, have received scant attention. The organ fats of animals as compared with their depot fats have been neglected.

The most recent comprehensive account of the distribution and occurrence of fatty acids is in Hilditch's (3) "Chemical Constitution of Natural Fats." Hilditch makes it evident that there is a broad pattern of fatty acid types and distribution, which fits into the phylogenetic scale and also follows a classification of species based on morphological grounds. This pattern shows the tendency of the more complex and highly developed forms of life to elaborate the simplest types of fat. However, in the animal world, as will be discussed later, fats are derived directly from the dietary fats as well as endogenously produced, so that the fats found in the body depend to some extent on those present in the diet.

Studies on the classification of species and the composition of the fats that they elaborate have been based to a large extent on the examination of fats from an individual specimen or from bulk samples comprising many individuals. Little attention has been paid to the possibility of variation between individuals within a given species. It has been shown that in some plants, such as flax (linseed), the seed oils are fairly characteristic of the species though occasional variations occur with different varieties of seed and in different climatic conditions. However, variants from the normal or usual type of fat have been found in seed fats. Although safflower (*Carthamus tinctorius*) seed oil normally has an iodine value of about 140–150 and contains about 10% saturated acids with 20% oleic and 70% linoleic acid, Horowitz and Winter (*4*) found two individual specimens among a large number of plants whose seed oils had iodine values of only 100 and 105. From these specimens a variant has been bred whose seed oil has an iodine value ranging from 91 to 101. Moreover, the fatty acid composition is changed from that of the normal seed oil and consists of saturated acids, 4–8%; oleic acid, 74–79%; and linoleic acid, 11–19%. The elaboration of different fatty acids within a given species of plant has not yet been observed, but the possibility cannot be overlooked in the light of observations on other constituents. Different strains of the same organism may elaborate different types of fatty acids. Thus the physiologically active α,β-unsaturated C_{27}-phthienoic acid is present in virulent strains of the tubercle bacillus but absent from the nonvirulent strains, which also show other differences in the composition of the higher branched-chain fatty acids (*5*).

While admitting broad differences between species in different parts of the phylogenetic scale, we have therefore to accept the possibility of occasional considerable variation in fatty acid composition between individuals of the same species and even the occurrence of specific fatty acids in one individual and their absence in another. Furthermore, studies on the occurrence of essential oils and alkaloids show that species which are adjudged to be the same on morphological grounds may elaborate different substances. A striking illustration of this is afforded by the work of Penfold and Morrison (*6*). This shows that the composition of the essential oil of eucalypts cannot be used, as earlier supposed by Baker and Smith (*7*), as an unequivocal guide to botanical classification. The first evidence of chemical variation was obtained in 1924 from specimens of *Eucalyptus dives*, which, though morphologically identical, elaborated in their leaves entirely different types of essential oils. Penfold and Morrison (*8*) distinguished three physiological forms within this species, each of which propagates its own form with the same characteristic essential oil. The variations in chemical composition are not altered by the environment but are inherent in the physiological form.

In the alkaloid field, examples of variations within a given species include the elaboration of hyoscine as the main alkaloidal constituent in some specimens of *Duboisia myoporoides* and of hyoscyamine in other specimens (*9*).

In one specimen of *Daphnandra micanthra* the bark alkaloids were found to consist mainly of daphnandrine and daphnoline with traces of micanthrine (*10*) whereas in another specimen micanthrine alone was present (*11*).

In microorganisms it is perhaps significant that the presence of strains in a particular culture may mean wide variations in the elaboration of constituents, as in penicillin-producing mold; or variations may be observed in the color of the microorganisms showing chemical modifications within a particular species (*12*).

In spite of the anomalies mentioned above, a study of chemical constituents can be useful as a guide to the classification of species. Bate-Smith (*13*) in considering the distribution of phenolic substances in plants has remarked: "It is therefore extremely encouraging to find the divisions of plants indicated by the pattern of distribution of phenolic substances so seldom at variance with the generally accepted systems of taxonomic classification." More recently Micks has used chromatographic techniques to assist in the solution of taxonomic problems in insects (*14*), particularly in regard to the classification of types of mosquitoes (*15*).

In the field of fats the evidence almost certainly points toward the occurrence of specific fatty acids in some species, especially in the vegetable kingdom, but the possibilities of variations within morphologically identical species cannot be overlooked.

In defining relationships between the occurrence of specific fatty acid types and the classification of species, it is desirable to eliminate the effect of environment. In animals, for example, it is obvious that the kinds and proportions of the fatty acids in the depot fats are influenced by those of the dietary fats. In extreme cases when the diet includes a high proportion of fat, the depot fats and the dietary fats may be very similar in composition. On the other hand, there is little evidence to show that wool or hair lipids are markedly changed by the diet or that the dietary fatty acids are incorporated into these lipids to any great extent. It is therefore possible that such lipid secretions are more characteristic of the species than are their depot fats.

Specific modifications may be imposed on the ingested fats that serve to characterize a class or a species. Thus it is found that in ruminants the dietary unsaturated fatty acids are partially hydrogenated, giving rise to *trans* and positional isomers of the ingested unsaturated fatty acids. These acids are not found to occur naturally elsewhere.

Information on the relationships between fatty acid occurrence and the classification of species is far from complete. The interpretation of our present knowledge requires an understanding of the metabolism of fatty acids and especially of their biosynthesis. Even in this field there are wide gaps and the formation of the unusual types of fatty acids found in some plants and microorganisms remains unexplained.

II. The Naturally Occurring Fatty Acids and Lipids

A. Types of Naturally Occurring Fatty Acids

The naturally occurring fatty acids may be divided into: (i) *n*-saturated acids; (ii) *n*-unsaturated acids; (iii) branched-chain acids; and (iv) cyclic acids.

1. *n-Saturated Fatty Acids*

The *n*-even-numbered acids from C_2 to C_{26}, inclusive, occur in natural fats, the most abundant being palmitic (C_{16}) acid. The higher members up to C_{36} are found in waxes. The presence of *n*-odd-numbered acids in certain animal (*16–18*) and plant fats (*19*, *20*) has only recently been satisfactorily established. All members of the series from C_3 to C_{25} have been isolated or detected. Substituted *n*-saturated acids are not commonly found in nature. However, optically active 2-hydroxy *n*-even-numbered acids from C_{12} to C_{18} together with 2-hydroxy-16-methylheptadecanoic acid have been isolated from wool wax (*21*), and the C_{24} and C_{26} homologs occur in brain lipids (*22*). In addition, ω-hydroxy acids (C_{18} to C_{30}) have been isolated from carnauba wax (*23*).

2. *n-Unsaturated Fatty Acids*

The unsaturated fatty acids are classified as: (*a*) monoethenoid, (*b*) diethenoid, (*c*) triethenoid, (*d*) polyethenoid, and (*e*) acetylenic acids. The double bonds of the naturally occurring fatty acids usually possess the *cis* configuration.

a. Monoethenoid Acids. Oleic (octadec-*cis*-9-enoic) acid is the most common of all fatty acids, but in the seed oils of the Umbelliferae it is largely replaced by petroselinic (octadec-*cis*-6-enoic) acid; and in lactobacilli, *cis*-vaccenic (octadec-*cis*-11-enoic) acid, but not oleic acid, is found (*24*). Other commonly occurring monoethenoid acids include palmitoleic (hexadec-*cis*-9-enoic) acid. Erucic (docos-*cis*-13-enoic) acid occurs in quantity in the seed fats of the Cruciferae. Ricinoleic (12-hydroxyoctadec-*cis*-9-enoic) acid is the best-known long-chain hydroxy acid, being present in high proportions (90%) in castor oil. 9-Hydroxyoctadec-12-enoic acid is present in the seed oils of *Strophan-*

thus sarmentosus (*25*) and *cis*-12,13-epoxyoctadec-*cis*-9-enoic acid occurs in quantity in the seed oils of *Vernonia anthelmintica* (*26*). All these oxygenated acids are optically active.

The naturally occurring *n*-even-numbered carbon monoethenoid acids range from C_{10} to C_{30}. In general the lower members are found in milk fats, those of intermediate range (C_{16}–C_{24}) in the oils of aquatic species, and the higher members in waxes, phosphatides, and unusual seed fats.

An odd-numbered acid, heptadec-*cis*-9-enoic acid, has been isolated from lamb caul fat, and evidence for the occurrence of a C_{15} unsaturated acid has been obtained (*27*). In the free fatty acids of human hair fat the full range of monoethenoid acids from C_{11} to C_{18} has been observed (*28*).

b. Diethenoid Acids. The most commonly occurring diethenoid acid is linoleic (octadeca-*cis*-9,*cis*-12-dienoic) acid, which is found in most seed fats. Other diethenoid acids include deca-*trans*-2,*cis*-4-dienoic acid found in *Stillingia* oil (*29, 30*).

c. Triethenoid Acids. Linolenic (octadeca-*cis*-9,*cis*-12,*cis*-15-trienoic) acid occurs in quantity in many vegetable oils. Other nonconjugated triethenoid acids include an isomer of linolenic acid, octadeca-6,9,12-trienoic acid, present in the seed fats of *Oenothera biennis* (*31*); a closely related C_{16} acid, hexadeca-7,10,13-trienoic acid occurs in rape leaf glycerides (*32*).

α-Eleostearic acid, octadeca-*cis*-9,*trans*-11,*trans*-13-trienoic acid, is the chief acid in tung oil and in some other drying oils; punicic acid, octadeca-*cis*-9,*trans*-11,*cis*-13-trienoic acid, which is isomeric with α-eleostearic acid, occurs in pomegranate seed oils (*33–35*). Other acids closely related to α-eleostearic, which occur in certain seed fats, include α-kamlolenic acid, which is 18-hydroxy-α-eleostearic (*36*) acid, and licanic acid, which is 4-keto-α-eleostearic acid (*37, 38*). Parinaric acid, which is octadeca-9,11,13,15-tetraenoic acid (*39*), contains two or three *trans* bonds (*40*).

d. Polyethenoid Acids. The best-known acid in this class, arachidonic (eicosa-5,8,11,14-tetraenoic) acid, is present in very small quantities in many animal fats and in larger amounts in the organ fats, especially in the phospholipid fractions. C_{20} and C_{22} unsaturated acids with four, five, or six double bonds also occur, especially in fish liver oils.

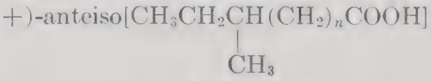
In animal fats these polyenoic acids are mainly of the linoleic type, that is, with the first double bond between the sixth and seventh carbons from the methyl group end, whereas in fish oils they are mainly of the linolenic type with the first double bond between the third and fourth carbons from the methyl group end (*41*). In all instances the double bonds are separated by single methylenic groups giving the structure

$$—CH{=}CH\cdot CH_2\cdot CH{=}CH—$$

e. Acetylenic Acids. Acetylenic acids are not commonly found, but have been isolated from the seed oils of a few species. Tariric (octadec-6-ynoic) acid is present in the seed oils of several species of *Picramnia.* [cf. Hilditch (*3*)] Erythrogenic (octadec-17-ene-9,11-diynoic) acid (*42*) has been found in the seed oil of *Onguekoa gore* Engler (= *Ongokea klaineana* Pierre). Ximenynic or santalbic (octadec-*trans*-11-en-9-ynoic) acid occurs in the seed fats of several *Ximenia* and *Santalum* species (*43–45*).

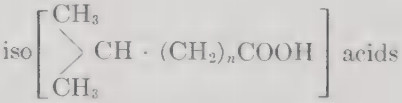
3. *Branched-Chain Fatty Acids*

Although the naturally occurring fatty acids are generally straight-chain compounds, the presence of branched-chain fatty acids is more widespread than was earlier believed. It has long been known that considerable amounts of isovaleric acid are present in the depot fats of the dolphin and porpoise, but it is only recently that branched-chain fatty acids have been found in animal fats, notably those of ruminants, as well as in vegetable fats. The branched-chain fatty acids isolated from ruminant fats consist of the odd series of



$$(+)\text{-anteiso}[CH_3CH_2\underset{\substack{|\\ CH_3}}{CH}(CH_2)_nCOOH]$$

acids from C_{13} to C_{17}, inclusive, and the odd and even series of



$$\text{iso}\left[\begin{matrix}CH_3\\ \\ CH_3\end{matrix}\!>CH\cdot(CH_2)_nCOOH\right]\text{acids}$$

from C_{13} to C_{18} inclusive. Acids with more than one methyl side chain have also been reported in ruminant fats (*46–48*). In wool grease, the range of (+)-anteiso[*] acids extends from C_9 to C_{31} (odd series only), and iso acids from C_{10} to C_{28}, only the even series being represented (*49*). The branched-chain fatty acids of tubercle bacilli and certain other acid-fast bacteria include tuberculostearic acid, phthioic acid, and mycolic acid. The first of these is known to be D-(—)-10-methylstearic acid (*50, 51*) the second is a mixture containing α,β-unsaturated branched-chain acids from which has been isolated mycolipenic acid (+)-2,4,6-trimethyltetracos-2-enoic acid (*52*) and mycoceranic acid (—)-2,4,6-trimethyloctacosanoic acid (*53*). The mycolic acids are branched-chain hydroxy acids. Their structure (I) is illustrated by that of α-mycolic acid Test (*54*).

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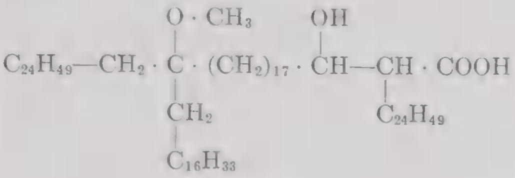
[*] The term *anteiso* was introduced by A. W. Weitkamp [*J. Am. Chem. Soc.* **67**, 447 (1945)] to indicate compounds with the methyl side-chain in the antepenultimate position.

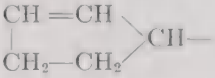


$$C_{24}H_{49}{-}CH_2\cdot \overset{O\cdot CH_3}{\underset{\underset{C_{16}H_{33}}{CH_2}}{C}}\cdot (CH_2)_{17}\cdot \overset{OH}{CH}{-}\underset{C_{24}H_{49}}{CH}\cdot COOH$$

(I)

4. *Cyclic Acids*

Acids containing cyclic systems are rare. In the lipids of lactobacilli, however, lactobacillic [ω-(2-*n*-hexylcycloprop-1-enyl)decanoic] acid with a cyclopropane ring system is a major component (*24*). The only other known fatty acid with a three-membered ring system is sterculic [ω-(2-*n*-octylcycloprop-1-enyl)octanoic] acid, which forms over 70% of the acids in the seed fat of *Sterculia foetida* (*55*). The seed oils of *Hydnocarpus* and a few other genera of the tropical family Flacourtiaceae, which are used to treat leprosy, have as major constituents, dextrorotatory fatty acids with a cyclopentenyl ring (R)



at the end of the fatty acid chain.

The best-known members are:

chaulmoogric,	$R\cdot(CH_2)_{12}\cdot CO_2H$
gorlic,	$R\cdot(CH_2)_6CH{=}CH\cdot(CH_2)_4CO_2H$
hydnocarpic,	$R\cdot(CH_2)_{10}CO_2H$

The constitution of chaulmoogric and hydnocarpic acids was established by Shriner and Adams (*56*) in 1925.

B. CLASSIFICATION OF LIPIDS

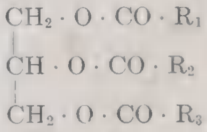
The classification and terminology of lipids have never been firmly settled. Even the spelling may vary from lipid to lipide or lipin, with or without a change in definition. In this chapter it is proposed to restrict the term lipid to the group of naturally occurring substances that yield on hydrolysis fatty acids, rather than to include other naturally occurring substances that are soluble in fat solvents, as is sometimes done by other authors.

In Bloor's classification of lipids (57) there are two main subdivisions—simple and compound—which denote, respectively, lipids containing C, H, and O only, and those which also contain P or N or both. A third subdivision, the "derived" lipids, is also given by Bloor; but as these are not generally present as such in living tissues, being derived by hydrolysis, they need not be considered here.

Simple lipids include fats, or triglycerides, and waxes. Fats are esters of glycerol and higher fatty acids, and waxes are esters of higher fatty alcohols and higher fatty acids. In this category may be included also sterol esters and vitamin A esters. Compound lipids contain other groups in addition to the alcohol, and in the subdivision of compound lipids there are various schemes and terminologies. The present author feels that the terms simple and compound do not serve any useful purpose in the classification of lipids and prefers to follow subdivisions based on the type of derivation as follows: (i) esters of glycerol (fats or triglycerides); (ii) esters of long-chain fatty alcohols and sterols (waxes); (iii) esters of glycerophosphoric acid coupled with a nitrogen base and/or a carbohydrate (phospholipids), and (iv) derivatives of the long-chain hydroxyamino alcohol sphingosine (sphingolipids).

1. *Fats*

These, as already indicated, are esters (II) of glycerol and higher fatty acids



$$\begin{array}{l} CH_2 \cdot O \cdot CO \cdot R_1 \\ | \\ CH \cdot O \cdot CO \cdot R_2 \\ | \\ CH_2 \cdot O \cdot CO \cdot R_3 \end{array}$$

(II)

where R_1, R_2, and R_3 are fatty acid radicals.

Living tissues contain little or no free fatty acid, and the glycerides present are triglycerides. However, monoglycerides occur in the hog pancreas (*58*). The naturally occurring fats as isolated are not purely triglycerides but contain unsaponifiable matter, including sterols, hydrocarbons, and alcohols. Related to the fats or triglycerides are the fatty esters of the α-glyceryl ethers of *n*-octadec-9-enyl (selachyl), *n*-octadecyl (batyl), and *n*-hexadecyl (chimyl) alcohols.

In natural fats the wide variety of fatty acids permits numerous combinations within the glyceride molecule. According to the views of Hilditch (*3*), no simple triglyceride is formed if any other combination is possible. This is the rule of even distribution, which, according to Hilditch, applies to most fats, although not with mathematical precision. To account for the relatively high content of fully saturated glycerides in many stearic acid-rich animal fats, Hilditch suggested that such fats are assembled on an even-distribution basis but are later modified in the animal by hydrogenation (*3*).

Other workers have suggested that a random (*59*, *60*) or a restricted-random (*61*) distribution operates. The evidence shows that the fatty acids are widely distributed among the glyceride molecules, but it is not possible to define precisely the nature of the distribution. However, the countercurrent extraction method as used by Scholfield and Hicks (*62*) has given promising results, which are interpreted to support an element of randomness in the biosynthesis of triglycerides.

More recently Mattson and Lutton (*63*) using pancreatic lipase, which specifically removes the fatty acids esterified with the primary hydroxyl groups of glycerol, have shown that the residual monoglycerides were selectively esterified.

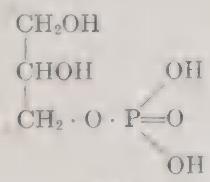
In vegetable oils the secondary hydroxyl group is esterified mainly with the unsaturated fatty acids, but in animal fats there is no general pattern of distribution except that of nonrandomization of the saturated and unsaturated fatty acids. These last results serve to emphasize the complexity of fatty acid distribution in the glyceride and afford the possibility of finding specific differences between species when sufficiently accurate data become available.

2. *Waxes*

These are usually defined as the higher fatty acid esters of the higher fatty alcohols $R \cdot CO \cdot O \cdot CH_2 \cdot R'$. In practice, however, natural waxes, as isolated, contain in addition many other components, including free fatty acids, free fatty alcohols, ketones, hydrocarbons, sterol esters, and sterols.

3. *Phospholipids and Related Substances*

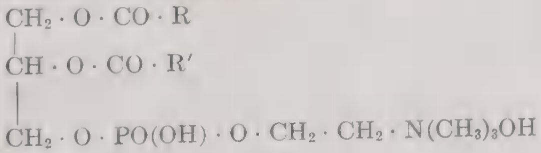
These may be regarded as derivatives of α-glycerophosphoric acid (III).



(III)

Normally the α- and β-positions are occupied by fatty acid radicals and the phosphoric acid radical is united through an ester linkage to a base such as choline, ethanolamine, or serine, or to the cyclic hexose inositol.

a. Phosphatidylcholine (Lecithin) (IV).



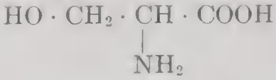
$$\begin{array}{l} CH_2 \cdot O \cdot CO \cdot R \\ | \\ CH \cdot O \cdot CO \cdot R' \\ | \\ CH_2 \cdot O \cdot PO(OH) \cdot O \cdot CH_2 \cdot CH_2 \cdot N(CH_3)_3OH \end{array}$$

(IV)

b. Phosphatidylethanolamine (*Cephalin*). Structure as in lecithin, but choline is replaced by ethanolamine,

$$HO \cdot CH_2 \cdot CH_2NH_2$$

c. Phosphatidylserine. Structure as in lecithin, but serine



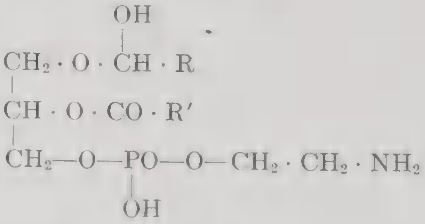
$$\begin{array}{l} HO \cdot CH_2 \cdot \underset{\displaystyle NH_2}{\underset{|}{CH}} \cdot COOH \end{array}$$

is substituted for choline.

In addition to the above, phospholipids without the nitrogenous base and designated phosphatidic acids (*64*) were isolated earlier from leaf tissues, such as those of cabbage. As, however, enzymes are present in these tissues which rapidly remove the nitrogenous bases to give phosphatidic acid, the occurrence in living tissues of any substantial amounts of this substance is doubtful (*65*).

In the phospholipids, lyso forms with one fatty acid radical in the α- or β-position are possible. It is well known that snake venom hydrolyzes fatty acids in the α-position only. Studies on the β-lyso forms of phosphatidylcholine and phosphatidylethanolamine of egg yolk have shown that, in accordance with the generally accepted view, the saturated acids are preferentially attached to the β-position (*66*). On the other hand, in milk glycerophospholipids unsaturated acids occur in both the α- and β-positions (*67*).

d. Plasmalogens. These are related to the phosphoglycerides, but the α-hydroxyl group is occupied by an aldehyde (jointed as an acetal) in place of a higher fatty acid (*68*) (V). Other evidence (*68a*) indicates that the aldehydogenic chain is bound to the secondary hydroxyl group of glycerol as an α,β-unsaturated ether.

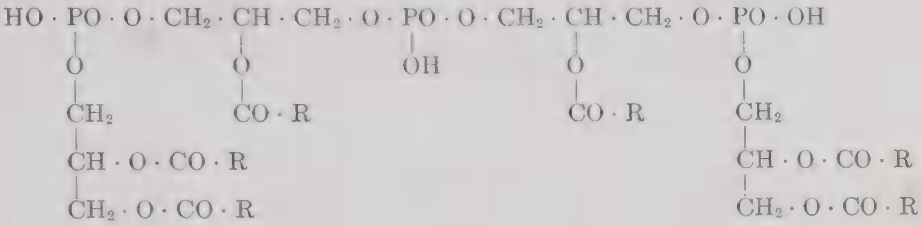


$$\begin{array}{l} CH_2 \cdot O \cdot \overset{\displaystyle OH}{\overset{|}{CH}} \cdot R \\ | \\ CH \cdot O \cdot CO \cdot R' \\ | \\ CH_2{-}O{-}\underset{\displaystyle OH}{\underset{|}{PO}}{-}O{-}CH_2 \cdot CH_2 \cdot NH_2 \end{array}$$

(V)

Plasmalogens also occur with choline attached to the phosphoric acid radical as in lecithin described above (*69, 70*).

e. Polyphosphoglycerides. This class is represented by cardiolipin, first isolated from heart muscle by Pangborn (*71*), who ascribed to it the structure shown (VI).

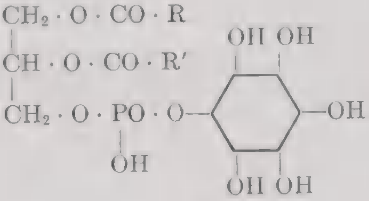


$$HO\cdot PO(O\cdot CH_2\cdot CH(O\cdot CO\cdot R)\cdot CH_2\cdot O\cdot CO\cdot R)\cdot O\cdot CH_2\cdot CH(O\cdot CO\cdot R)\cdot CH_2\cdot O\cdot PO(OH)\cdot O\cdot CH_2\cdot CH(O\cdot CO\cdot R)\cdot CH_2\cdot O\cdot PO(O\cdot CH_2\cdot CH(O\cdot CO\cdot R)\cdot CH_2\cdot O\cdot CO\cdot R)\cdot OH$$

(VI)

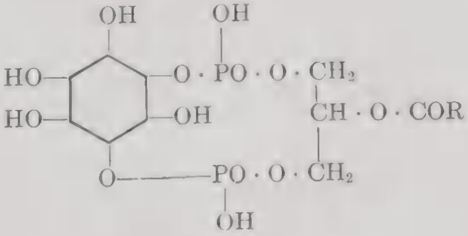
Macfarlane and Gray (72, 73) have since suggested a simpler structure for cardiolipin with two phosphoric acid residues, three glycerol residues, and four fatty acid residues.

f. Phosphoinositides. In this group are included a variety of phospholipids the structure of which has not been firmly established. The simplest example in this group is phosphatidylinositol, isolated from liver as well as from yeast (74). The constitution shown (VII) has been substantiated by later investigations (75).



(VII)

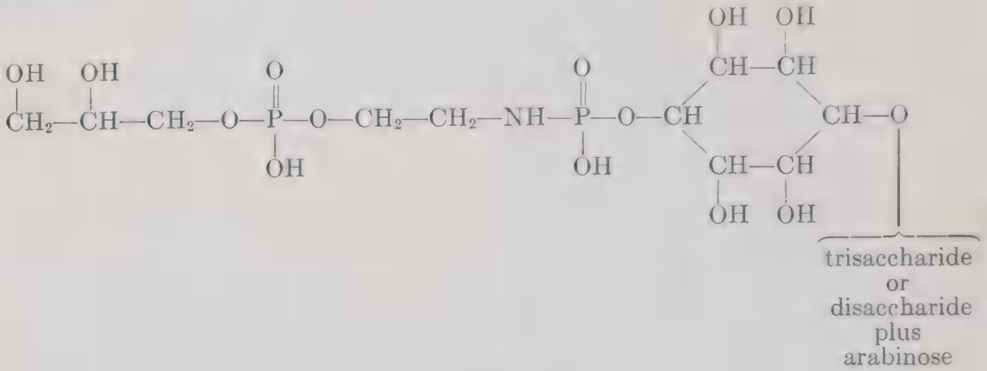
Diphosphoinositide was first isolated from brain tissue by Folch (76). The structure (VIII) of this phospholipid, was tentatively suggested by Hawthorne (77).



(VIII)

Malkin and Poole (78) isolated from commercial groundnut lecithin a phosphoinositide containing glycerophosphoric acid, inositol monophosphate, and ethanolamine in equal proportions, with three sugar molecules and two fatty acids to each glycerol molecule.

Of the two structures suggested, Folch and Le Baron (79) consider the one shown (IX) as more likely to be the correct one. The alternative structure would require inositol diphosphate among the products of hydrolysis, but this substance was not found.



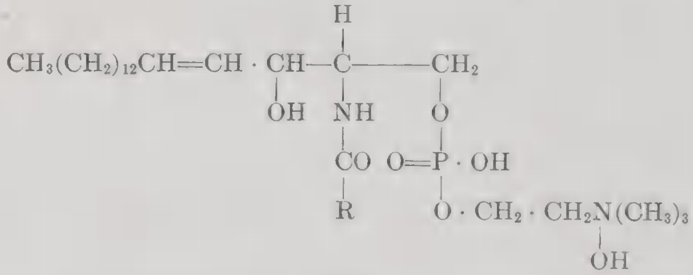
(IX)

4. *Sphingolipids*

These compounds are derivatives of the base sphingosine:

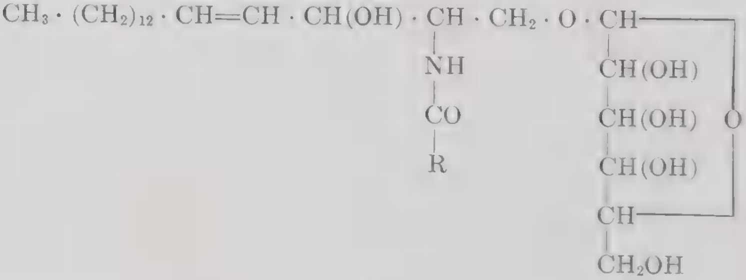
$$CH_3(CH_2)_{12} \cdot CH{=}CH \cdot CH(OH) \cdot CH(NH_2) \cdot CH_2 \cdot OH$$

In sphingomyelin (X) the amino group is attached to a fatty acid, lignoceric acid, and the terminal alcoholic group to phosphorylcholine.



(X)

In cerebrosides the terminal alcoholic group of sphingosine is united with galactose (or other hexose), and the fatty acid radical is united as the amide (XI).



(XI)

III. Formation of Fatty Acids and Lipids in the Living Organism

A. GENERAL CONSIDERATIONS

A similar mechanism of fatty acid synthesis involving the successive addition of active acetate or acetyl coenzyme A (acetyl CoA) units to form the long-chain fatty acids, as outlined in the Lynen fatty acid cycle (*80*), operates in both plants and animals. It may be expected, therefore, that similar types of fatty acids, including particularly palmitic, oleic, and, to a lesser extent, stearic acids will be found in both.

The presence in plants of a wider range and a greater diversity of fatty acids than in animals suggests that plants are provided with more varied or less specific enzymes. An explanation has still to be found for the formation of linoleic and linolenic acids in plants, but not in animals. Explanations are similarly required for the formation of specific hydroxy acids, cyclic acids, and acids containing acetylenic bonds. It has been suggested that lactobacillic acid with a cyclopropane ring is formed from *cis*-vaccenic acid by the addition of a methylenic group across the double bond (*24*). If this occurs in lactobacillus species why does it not occur generally in plants and animals?

B. FORMATION OF FATS IN ANIMALS

The fats of animals are derived: (*a*) endogenously by synthesis from the nonfatty constituents of the diet; and (*b*) exogenously from the fats in the diet, with or without modification.

1. *Endogenous Fat*

It has long been known that animals on low fat-content diets deposit more fat than is present in the diet and that when fat-free diets are used, carbohydrates or proteins give rise to similar fats, as shown in Table I.

It will be seen from Table I that endogenous fat consists essentially of C_{16} and C_{18} acids. Palmitic acid is fairly constant at 25–30%, and the unsaturated acids are largely monoethenoid. The traces of highly unsaturated C_{20} and C_{22} acids originate from the dietary linoleic and linolenic acids by the addition of acetate to the carboxyl end followed by desaturation. Linoleic acid gives rise to acids with the first double bond at carbon 6 from the methyl group end (linoleic type), and linolenic acid forms the linolenic type with the unsaturation commencing at carbon 3 from the methyl group end (*84, 85*).

The mechanism of the formation of endogenous fat has only recently been satisfactorily elucidated.

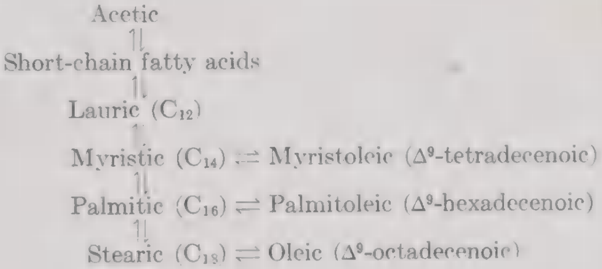
TABLE I

THE FATTY ACID COMPOSITION (MOLES %) OF THE DEPOT FAT OF ANIMALS ON A "FAT-FREE" DIET

Species	Diet	Saturated					Unsaturated				
									C_{18}		
		C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	Oleic	Lin.[a]	C_{20}–C_{22}
Rat, total fat *(81)*	Sucrose	—	3.1	26.7	3.6	0.4	0.9	15.6	47.2	2.2	0.3
	Casein	—	2.8	29.7	3.8	1.1	1.0	15.6	43.8	2.0	0.2
Pig											
Outer back *(82)*	Skim milk	—	2.5	28.9	9.8	—	—	5.3	53.4	0.1	—
Inner back	Skim milk	—	3.1	29.2	11.9	—	—	5.9	49.3	0.5	0.1
Sheep											
External tissue *(83)*	Wheat straw	0.4	4.4	25.9	23.6	—	0.4	0.8	42.5	1.5	0.5
Perinephric	Wheat straw	—	3.3	24.7	36.6	—	0.2	1.1	31.2	2.1	0.8

[a] Lin. = calculated as octadecadienoic acid but probably includes some octadecatrienoic acid.

Using isotopically labeled acetate, Rittenberg and Bloch (*86, 87*) showed that acetate is a precursor of the higher fatty acids, the condensation probably taking place through the successive addition of active acetate to the carboxyl group of the fatty acid; or, as has been subsequently shown, the condensation of the acetate unit with the fatty acids takes place through their CoA derivatives. This is the reverse of β-oxidation, which was first demonstrated by Knoop (*88*) in 1905. By use of isotopically labeled compounds, interrelationships in the synthesis and degradation of fatty acids were established (reaction sequence 1) [cf. Shorland (*89*)].


Acetic
Short-chain fatty acids
Lauric (C_{12})
Myristic (C_{14}) ⇌ Myristoleic (Δ^9-tetradecenoic)
Palmitic (C_{16}) ⇌ Palmitoleic (Δ^9-hexadecenoic)
Stearic (C_{18}) ⇌ Oleic (Δ^9-octadecenoic)

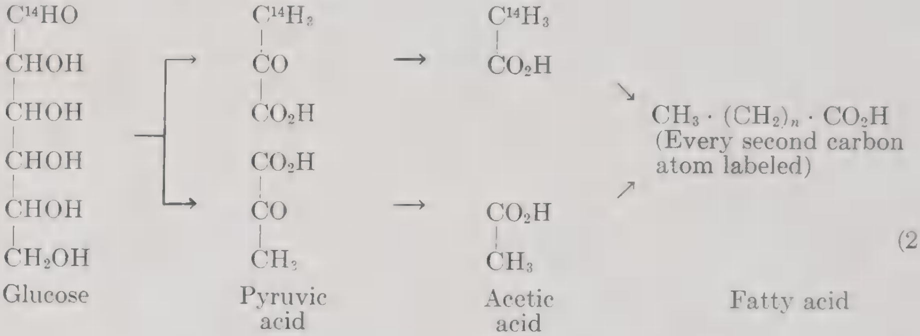
(1)

One of the difficulties in accepting acetate as the precursor in the production of endogenous fat has been the apparent absence of acids below C_{10} in animal depot fats. Now, however, more sensitive methods have revealed their presence (*90*). Moreover, it has since been demonstrated that the mammary gland possesses extraordinary synthetic activity, giving rise, particularly in ruminant milk fats, to the full range of *n*-even-numbered carbon fatty acids from C_2 to C_{16} [cf. Popjak (*91*); Folley (*92*)]. In ruminants the products of digestion in the rumen consist largely of acetate, which is transported in the blood stream to the udder, and so the sequence of events in fat formation can be visualized.

After the intravenous injection of labeled acetate into a lactating goat (*93*), a relationship was found between specific activity and chain length. The mechanism of the synthesis was further confirmed by the degradation of the individual fatty acids.

It has become clear that the conversion of protein and carbohydrates to fats involves their prior degradation to acetate. After the administration of labeled pyruvate, glucose, and starch to lactating rabbits, the degree and distribution of the labeling in the milk fatty acids showed that the test substances were transformed to acetate prior to their conversion to fatty acids (*94*). The conversion to acetate of the constituents mentioned was confirmed by excretion experiments involving the use of *p*-aminobenzoic acid according to the technique described by Bernhard (*95*).

The behavior of pyruvate indicated that the conversion to acetate took place through decarboxylation. As glucose was known to be broken down in the glycolytic cycle so that carbons 1 and 6 became the methyl carbons of pyruvic acid, it was possible to trace the route followed by glucose in its conversion to fat. Besides acting as a source of acetate, glucose and starch have been shown to assist fat synthesis by providing the glycerol required for the formation of triglycerides (*96*) (reaction sequence 2).



(2)

Similarly, certain amino acids, such as leucine (*97*) and alanine (*98*), are converted to acetate in animals, while others, such as glutamine, are well-known glycogen formers. Thus a pathway for the conversion of protein to fats is provided.

The observations recorded above indicate that acetate is the building stone of fat. Acetate per se is not active, however, and it was not until 1951 that the active acetate unit was identified by Lynen and Reichert (*99*, *100*) as the thioester of coenzyme A and acetic acid.

Acetyl CoA has been shown to be produced through the action of enzyme-bound adenosine triphosphate (ATP or Ad · P ∼ PP), CoA, and acetate as shown in reaction sequence 3.

$$\begin{aligned} \text{Enzyme} + \text{Ad}\cdot\text{P} \sim \text{PP} &\rightleftharpoons \text{Enzyme—P}\cdot\text{Ad} + \text{PP} \\ \text{Enzyme—P}\cdot\text{Ad} + \text{CoA}\cdot\text{SH} &\rightleftharpoons \text{Enzyme—S}\cdot\text{CoA} + \text{Ad}\cdot\text{P} \\ \text{Enzyme—S}\cdot\text{CoA} + \text{Acetate} &\rightleftharpoons \text{Enzyme} + \text{Acetyl—S}\cdot\text{CoA} \end{aligned} \qquad (3)$$

The mechanism outlined operates in tissues, yeast, and some microorganisms (*101*). As will be mentioned later, in other microorganisms another enzymatic pathway exists.

The attachment of the sulfhydryl group of coenzyme A to the acetyl group confers on it the properties of an acid as well as labilizing the methyl hydrogen. With such head and tail labilization of the acetate molecule it is possible to visualize the chemical mechanisms of the biological synthesis in terms of Lynen's fatty acid cycle (*80*) (Fig. 1).

The extension of the fatty acid molecule by two carbon units is achieved in four consecutive reactions, catalyzed by separate enzymes as follows: (*a*) One molecule of acetyl coenzyme A condenses with another giving acetoacetyl coenzyme A. (*b*) Acetoacetyl coenzyme A is reduced to β-hydroxybutyryl coenzyme A in the presence of reduced diphosphopyridine nucleotide. (*c*) β-Hydroxybutyryl coenzyme A undergoes degradation to crotonyl coenzyme A. (*d*) Crotonyl coenzyme A is reduced to butyryl coenzyme A in the presence of reduced flavin. The repetition of the cycle, eight times in a spiralwise manner, will produce stearyl CoA. The reaction between fatty acid CoA and acetyl CoA requires specific enzymes, according to the chain length of the fatty acid. One of these enzymes activates acetate and propionate (*102*); another, C_4 to C_{11} acids (*103*); and a third, higher fatty acids up to C_{22} (*104*). For each of the intermediate steps in the cycle there have similarly been found several different reducing enzymes, each with varying degrees of specificity [cf. Lynen (*105*)].

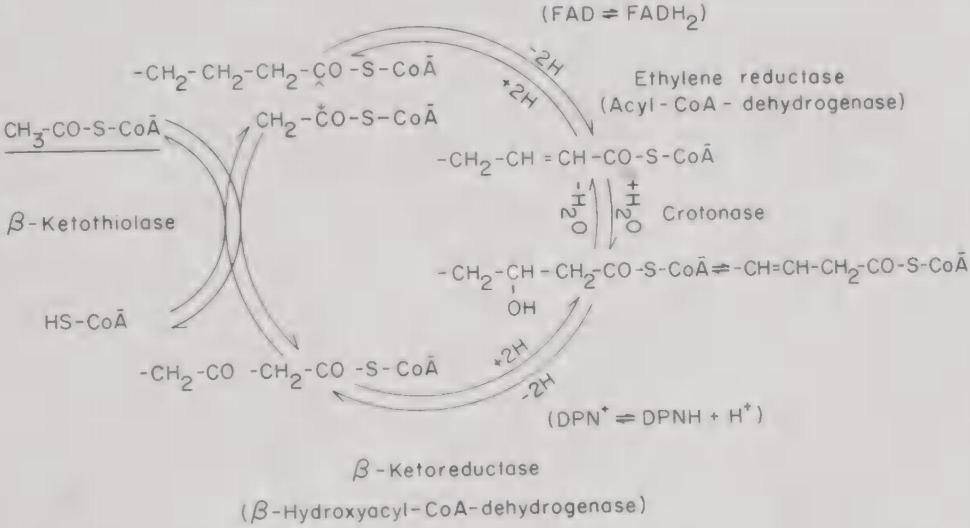


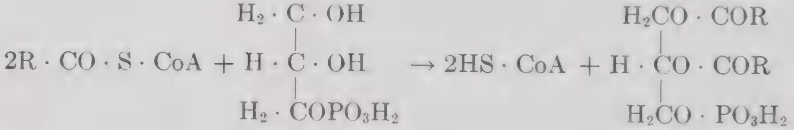
FIG. 1. The fatty acid cycle according to Lynen (*80*).

The reactions outlined in the cycle are reversible. Acetyl coenzyme A may condense with oxalacetate to enter the citric acid cycle, where it is broken down to carbon dioxide and water. The rate of oxidation depends on the availability of oxalacetate. If the availability is reduced through depression of carbohydrate metabolism, as in diabetes, then acetyl CoA cannot be metabolized completely and acetoacetyl CoA accumulates.

For the synthesis of fat there is needed a supply of acetyl CoA and reduced diphosphopyridine nucleotide (DPNH) derived from the breakdown of carbohydrate. A high concentration of these substrates must be maintained, and the concentration of free CoA kept low, if the equilibria of β-ketothiolase and of β-ketoreductase are to be shifted in the direction of synthesis.

It appears that the complex enzyme system that catalyzes the oxidative degradation, but not the synthesis, of fatty acids is localized in the mitochondria of the liver cell and that the particle-free soluble extracts of the liver and mammary gland, when properly supplemented, catalyze the synthesis of fatty acids [cf. Kennedy (*106*)].

The synthesis of glycerides and phospholipids may now be considered. Kornberg and Pricer (*104*, *107*) found that the sediment of the liver homogenate of the guinea pig possessed an enzyme capable of synthesizing the phosphatidic acids from acyl CoA and α-glycerophosphoric acid (4).



$$2R\cdot CO\cdot S\cdot CoA + \begin{array}{l} H_2\cdot C\cdot OH \\ \quad | \\ H\cdot C\cdot OH \\ \quad | \\ H_2\cdot COPO_3H_2 \end{array} \rightarrow 2HS\cdot CoA + \begin{array}{l} H_2CO\cdot COR \\ \quad | \\ H\cdot CO\cdot COR \\ \quad | \\ H_2CO\cdot PO_3H_2 \end{array} \qquad (4)$$

This enzyme has a distinct optimum with coenzyme A derivatives of C_{16}–C_{18} acids. The preferential occurrence of C_{16}–C_{18} acids in the body might thus be explained.

Phosphatidic acids are not found in fresh tissues, probably owing to their rapid dephosphorylation to form orthophosphate and D-1,2-diglyceride. An enzyme concerned with this reaction was shown to be present in chicken liver(*108*). The D-1,2-diglyceride thus produced may then react with a long-chain fatty ester of CoA to form triglyceride, as described above, or else react with cytidine diphosphate choline to form lecithin (*109*), or cytidine diphosphate ethanolamine to form phosphatidyl ethanolamine (*110*).

Sphingomyelin is similarly formed by the transfer of the phosphorylcholine moiety of cytidine diphosphate choline to the free primary hydroxyl group of lignocerylsphingosine (*111*). On the other hand, inositol phosphatides are considered to result from the reaction of cytidine diphosphate D-α,β-diglycerides with the hydroxyl group of inositol (*112*).

The close link between the synthesis of glycerides and phospholipids is thus established, and it may be anticipated that phospholipids and triglycerides in the same tissues will have similar fatty acid compositions. As will be later mentioned (Section V), however, the distribution of the types of fatty acids between glyceride and phospholipids and between phospholipid species shows widely different patterns. There is

TABLE II

Modification of the Composition of Depot Fat by Feeding Dietary Fat

		Fatty acids (moles %)											
		Saturated						Unsaturated					
											C_{18}		
Fat	Diet	Below C_{12}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{12}	C_{14}	C_{16}	Oleic	Lin.[a]	C_{20}
Rat (*81*), total fat	Sucrose	—	—	3.1	26.7	3.6	0.4	—	0.9	15.6	47.2	2.2	0.3
Rat (*113*), total fat	Corn oil (84% total calories)	—	—	0.7	12.2	2.9	0.2	—	—	6.9	44.8	32.3	—
Corn oil (*113*)	—	—	—	1.7	11.0	2.9	—	—	—	1.6	48.8	34.0	—
Rat (*114*), total fat	Coconut oil (84% total calories)	0.7	31.8	18.1	18.6	2.7	0.6	0.6	1.5	4.1	20.1	1.2	—
Coconut oil (*114*)	—	19.4	47.2	16.4	8.5	1.7	0.2	—	—	1.1	5.5	tr.	—

[a] Lin. = calculated as octadecadienoic acid but probably includes some octadecatrienoic acid.

thus evidence of enzyme selectivity during synthesis of these lipids or of subsequent acyl interchange.

2. *Exogenous Fat*

There are many experiments to show that the dietary fat exerts a profound influence on the composition of the depot fats. In extreme cases when the diet includes considerable proportions of fat, the fatty acid composition of the dietary fat and the depot fat may be almost indistinguishable, as shown in Table II. Thus rats fed corn oil deposit fat containing similar proportions of fatty acids to those found in the diet.

Sometimes there are complicating factors so that the effect of the dietary fat is not so obvious as is illustrated by the fatty acid composition of the depot fats of rats fed coconut oil (see Table II). The lower fatty acids are here shown to be preferentially metabolized. In general it is found that the lower fatty acids (below C_{12}) are not stored in the depot fats of animals. However, dietary octanoic and decanoic acids are stored in the skin of rats (*115*).

Although the concept of the dynamic state (*116*) has been applied to the endogenous fatty acids of depot fats, it appears that the dietary fatty acids that are not formed endogenously, including linoleic, linolenic, and highly unsaturated C_{20}–C_{22} acids, are not readily mobilized and tend to remain in the depot fats after withdrawal from the diet [cf. Longenecker (*113*)]. Dietary (+)-14-methylhexadecanoic acid similarly persists in rat depot fats after withdrawal from the diet (*117*).

In ruminants the dietary unsaturated acids are hydrogenated in the rumen (*118–120*) with the result that these acids, notably linoleic and linolenic, are not found to any appreciable extent in the depot fats, but are largely converted to stearic acid and to *cis* and *trans* positional isomers of oleic acid (*121*).

C. SYNTHESIS OF FATS IN HIGHER PLANTS

The wider range of fatty acids in lipids of higher plants suggests that there must be available mechanisms for synthesis in addition to those found in animals. It appears that, like the higher animals, the higher plants transform carbohydrates and possibly proteins into fats, though the experimental evidence is perhaps less definite than might be desired.

The earlier studies on fat metabolism in plants, which have been reviewed by Hilditch (*3*), show that during the ripening of seeds there is a decrease in carbohydrate content accompanied by an increase in the fat content. The increase in iodine value of the oil during ripening was attributed to desaturation of the fatty acids.

Grindley, using cottonseed (*122*), was the first to describe the quantities of fatty acids and other constituents on a per seed basis. In these seeds saturated, oleic, and linoleic acids all continue to increase in weight during ripening. Any increase in unsaturation may thus be interpreted as an increase in synthesis of unsaturated acids rather than a desaturation of saturated acids. Similar results were obtained by Simmons and Quackenbush (*123*), using Lincoln soybeans. No evidence for dehydrogenation of saturated fatty acids was found either in the oil analyses or in the tests of the tissues for dehydrogenase activity. In another experiment excised stems bearing pods and leaves supplied with C^{14}-labeled sucrose were used (*124*). After 2 days 56% of the total radioactivity was present in oleic acid, which constituted about one-third of the total, while linoleic acid, which was also present in similar amounts, contained but 18% of the total activity. Five days after withdrawal, the total activity in the oleic fraction doubled, linoleic showed a fourfold increase, and linolenic a sevenfold. Since the relative percentages of different fatty acids had not changed, the evidence, contrary to that earlier obtained, suggested the possibility of some conversion of oleic acid to the other acids.

Climatic conditions have been found to influence the content of more unsaturated fatty acids, as shown by Painter and Nesbitt (*125*) for linseed oil. Similar observations have been made on other unsaturated oils, notably sunflower seed oils. Using seeds taken from field trials in diverse regions of the Australian Commonwealth, Budge and co-workers (*126*) found that the oils from the Northern Territory contained 31–36% linoleic acid as compared with 65% in the most southerly latitudes.

The mechanism of the formation of fatty acids in higher plants has not been studied so extensively as in animals. However, Newcomb and Stumpf (*127*) showed that slices of peanut cotyledons synthesize radioactive long-chain fatty acids from C^{14} acetate. Subsequently Gribble and Kurtz (*128*) made *in vitro* cultures of developing flax seeds with the addition of acetate-1-C^{14}. The individual fatty acids, including palmitic/stearic, oleic, linoleic, and linolenic, when degraded showed much more radioactivity in first and third carbon atoms than in the second and fourth. The results are thus consistent with those for animal tissues in respect of the formation of fatty acids by "head to tail" condensation of acetate.

Further evidence of the nature of the fatty acid synthesis in higher plants has been obtained by Stumpf and Barber (*129*), who found that particles from avocado fruit mesocarp incorporate C^{14}-labeled acetate into esterified long-chain fatty acids. ATP, CoA, and Mn^{2+} are essential components of the system, and palmitic and oleic acids were the only labeled acids that accumulate. It appears that fatty acid formation in the avocado is by condensation of CoA-activitated acetate units. The evidence supports the concept that as in animals the biosynthesis of fatty acids in the avocado is through a mechanism essentially the reverse of β-oxidation, which is known to occur in plants. Stumpf and Barber (*130*) found, for example, that the mitochondria from peanut cotyledons possessed a fatty acid oxidase system similar to that present in animal tissues. The acetyl coenzyme A thus produced is similarly oxidized via the Krebs cycle and some accumulation of acetoacetate also occurs. However, in addition, in higher plants, other mechanisms are present for the oxidation of fatty acids. One of these is associated with the microsomal particles and catalyzes the oxidation of palmitate along the carbon chain with the aid of DPN (*131*). Another, present in the soluble fraction after removal of mitochondria and microsomes, oxidizes only the carboxyl group of palmitic acid (*132*).

The mechanism for the interconversion of fat and carbohydrates in plants has not been generally established. However, Beevers (*133*) has shown that cell-free extracts of endosperm and cotyledons of castor bean seedlings bring about the production of malate from isocitrate and acetate, thereby providing a mechanism for the interconversion of fat and carbohydrates.

D. Synthesis of Fats in Microorganisms

It is well known that yeasts and molds when grown on a glucose or sucrose medium produce much fat, thus showing that, as in animals, a mechanism exists for the conversion of carbohydrates to fats. The possibility of the participation of acetate units in this conversion is indicated from early experiments with timothy grass bacillus (*Mycobacterium phlei*) in which it was shown that the addition of acetate to the medium increased the amount of lipid formed (*134*). The first definite proof, however, of the participation of acetate in the synthesis of the lower fatty acids in microorganisms was that of Wood *et al.* (*135*), who showed in 1945 that *Clostridium acetobutylicum* synthesizes butyric acid from two molecules of acetate. Subsequently studies on *C. kluyveri* shed much light on the mechanism of fatty acid synthesis. This anaerobic organism requires for food only ethanol and acetate, which it converts almost quantitatively to butyric and caproic acids. The building up of the higher fatty acids does not take place, as the coenzyme A transphorase system will not catalyze the activation of fatty acids with more than eight carbon atoms (*136*).

The main interest in the studies of fatty acid synthesis of *C. kluyveri* is the demonstration of a fatty acid cycle involving the participation of acetyl coenzyme A as in the Lynen cycle, but differing from it in that the regeneration of coenzyme A does not take place through the citric acid cycle. Instead, the activation of acetate is initiated by the phosphorylation of acetate (*101, 137*)

$$\text{Acetate} + \text{ATP} \rightleftharpoons \text{Acetyl phosphate} + \text{ADP}$$

followed by the acetyl transfer through transacetylase to coenzyme A:

$$\text{Acetyl phosphate} + \text{CoA} \rightleftharpoons \text{Acetyl CoA} + \text{Phosphate}$$

Bernhard and associates (*138*) grew the mold *Phycomyces blakesleeanus*, which produces a wide range of fatty acids, on a glucose medium to which was added C^{14}-labeled acetate. At the beginning γ-linolenic (octadeca-6,9,12-trienoic) acid was the most strongly labeled, but the activity thereafter slowly decreased. The C_{22}, C_{24}, and C_{26} acids showed an increased activity as the experiment proceeded, reaching a maximum at 4 days, and then the activity diminished slowly toward the end of the experiment, which lasted 8 days. The activity of stearic acid was low throughout, whereas that of palmitic acid tended to increase. The activity of oleic and of linoleic acid remained high, the activity of latter tending to increase toward the end of the experiment.

Further experiments (*139*) showed that the percentage of activity of each of the fatty acids remained constant during growth and that no hydrogenation of linoleic or linolenic acid to saturated acids therefore took place.

The results as a whole do not suggest the conversion of oleic into the more highly unsaturated constituents; they therefore confirm generally the observations made on seeds.

IV. Relationships between Types and Distribution of Fatty Acids and Their Biological Origin

A. INTRODUCTION

In this section it is necessary, because of the limited data, to base conclusions mainly on the composition of the triglycerides in the depot fats of different species. Occasionally, however, the lipid constituents are mainly or wholly nonglyceridic as in *Mycobacterium tuberculosis* or in the seed lipids of the shrub *Simmondsia californica*, and so comparisons between the types and distribution of fatty acids cannot always be made on a uniform basis. In this section, for the most part the

TABLE III

THE COMPOSITION (WEIGHT %) OF ANIMAL DEPOT FATS

Species	Saturated				Unsaturated						
							C_{18}				
	C_{14} (and below)	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	Monoene	Diene	Triene	C_{20}	C_{22}
Aquatic animals											
Fish, freshwater[a]	4	13–15	2	—	1	ca. 20	—	40–45	—	ca. 12	0–5
Fish, marine[a]	3	12–15	2	—	1	15–18	—	27–30	—	20–25	8–12
Whales[a]	7	15–18	2	—	1–2	15–18	—	35–40	—	15–20	5–10
Amphibians and reptiles											
Frog[a]	4	11	3	—	—	15	—	52	—	15	
Turtle (*141*)	10.3	15.0	7.2	1.2	1.3	12.9	—	23.9	—	20.7	4.6[b]
Python (*142*)	1.3	19.7	10.8	1.2	0.5	3.9	47.0	10.7	0.8	4.1	
Crocodile (*143*)	3.3	26.6	4.8	0.6	1.3	6.5	33.5	17.0	2.6	3.8	
Birds											
Ostrich (*144*)	0.9	24.8	5.9	0.4	0.9	6.1	39.8	17.1	3.8	0.3	
Flamingo (*145*)	—	24.8	7.7	—	—	4.9	53.4	7.1	0.2	1.9	
Mammals											
Rodents											
Mouse (*146*)	0.2	26.7	2.6	—	—	5.6	35.8	26.2	1.9	1.0	
Porcupine (*146*)	5.2	36.3	11.7	—	1.5	4.1	27.1	13.6	0.5	—	
Herbivora											
Pig (*147*)	1.3	28.3	11.9	—	0.2	2.7	47.5	6.0	—	2.1	
Hippopotamus (*148*)	2.3	27.1	22.2	1.1	0.4	2.2	39.3	3.5	1.5	0.4	
Carnivora and Omnivora											
Puma (*149*)	4.1	24.2	10.5	0.8	0.9	4.6	39.5	8.6	3.6	3.2	
Chimpanzee (*150*)	2.8	29.8	6.8	0.2	1.1	5.1	44.5	8.0	—	1.7	

[a] Data from T. P. Hilditch and J. A. Lovern (*140*), except that in the aquatic animal fats the amounts of C_{14}, C_{18} saturated acids and C_{14} unsaturated acids have been added by the writer.

[b] 2.9% of C_{24} acids of mean unsaturation -10.0 H was present. The mean unsaturation of the C_{20} and C_{22} acids was -4.3 H and 5.0 H; this is somewhat lower than that of fats of aquatic animals.

comparisons between the fatty acids of different species will be concerned with the main fatty depots.

It is convenient when making comparisons between fatty acids in the lipids of different species to commence with the fats of aquatic origin, to proceed to those of land animals, and finally to the fats of vegetable origin. As noted by Hilditch and Lovern (*140*), there is a gradual simplification in fatty acid composition as we proceed from the simpler to the more highly organized forms of life. In Table III the decreasing complexity of fatty acid composition—from aquatic species through amphibians, reptiles, birds, and mammals—parallels, to some extent, evolutionary development. Although the present author (*151*) regards the fats of the diet as being largely responsible for this, nevertheless it is convenient to deal with the animals in the order outlined and to separate aquatic species from land animals and land plants, each group of which has its own characteristic differences in respect of types and distribution of fatty acids in its lipids.

B. Fats of Aquatic Species

The fats of aquatic species are characterized by the low content of saturated acids (mainly palmitic acid), by their wide range of unsaturated fatty acids, and especially by the large amounts of C_{20} and C_{22} highly unsaturated acids. The last-mentioned also occur in the organ phospholipids of land mammals but are present only in traces in their depot fats. As in land animals the C_{16} and C_{18} unsaturated acids are mainly monounsaturated.

Apart from traces of linoleic acid, the polyenoic acids of aquatic fats are shown to be predominantly of the linolenic type, though very small amounts of linolenic acid itself are present. The most abundant C_{18} polyenoic unsaturated acid is stearidonic (octadeca-6,9,12,16-tetraenoic) acid, not found in the lipids of terrestrial plants and animals. The fats of terrestrial animals, on the other hand, contain, as a rule, both linoleic and linolenic types, the former being more abundant (*41*) (see Table IV).

TABLE IV

THE UNSATURATED FATTY ACIDS OF AQUATIC AND TERRESTRIAL SPECIES (*41*)

Acid	Aquatic	Terrestrial
C_{18}	$\Delta^{6,9,12,15}$	$\Delta^{9,12}$ and $\Delta^{9,12,15}$
C_{20}	$\Delta^{5,8,11,14,17}$ with some $\Delta^{5,8,11,14}$	Mainly $\Delta^{5,8,11,14}$
C_{22}	$\Delta^{4,7,10,13,16,19}$ with some $\Delta^{7,10,13,16,19}$	Mainly $\Delta^{7,10,13,16,19}$, less $\Delta^{4,7,10,13,16,19}$

TABLE V

THE FATTY ACID COMPOSITION (WEIGHT %) OF THE LIPIDS OF SPONGES (*155*)

	Saturated					Unsaturated							
	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}	C_{28}
Spheciospongia vesparia	1.7	9.1	0.7	—	—	0.4 (2.0 H)	3.1 (2.0 H)	15.9 (2.0 H)	6.5 (>2.0 H)	4.4 (>2.0 H)	4.5 (>2.0 H)	38.8 (>2.0 H)	14.9 (>2.0 H)
Suberites compacta	0.3	6.6	1.0	1.1	0.6	0.2 (2.0 H)	3.1 (2.0 H)	11.1 (2.3 H)	7.8 (2.3 H)	17.8 (6.3 H)	11.6 (7.3 H)	21.6 (>2.0 H)	17.0 (4.5 H)

1. *Fats of Aquatic Flora*

Lovern (*152*) has found that the fats of marine or freshwater species, including those of algae and higher plants so far examined, resemble somewhat those of freshwater animals. As noted by Hilditch (*3*), there is an indication in the algae fats that the compositions fall into groups agreeing with their botanical relationships, irrespective of their habitat (freshwater or marine). The fat of the red alga alone has C_{20} and C_{22} unsaturated acids in amounts corresponding to those of marine animals. There has been no detailed analysis in regard to the nature of the types of unsaturated acids, but Paschke and Wheeler (*153*) found that C_{18} unsaturated acids of the freshwater chlorella consisted mainly of linolenic acid, with lesser proportions of linoleic and monoethenoid acids. The tetraenoic acids formed only a minor proportion. This appears to contrast with the fats of marine animals, in which the polyenoic acids are largely tetraenoic (stearidonic) acid.

2. *Fats of Aquatic Invertebrates and Differentiation into Marine and Freshwater Types*

Although the fats of aquatic plants show no marked differences between freshwater and saltwater species, the crustacea feeding on marine phytoplankton possess the marine type of fat, which then persists throughout marine animal life (*154*). The differences in the composition between the fats of marine and freshwater species are probably of dietary origin, as will be discussed later.

In most instances, the fats of marine and freshwater species conform fairly closely in composition to the average marine and freshwater types indicated in Table III. It will suffice, therefore, to mention a few of the exceptions. The few observations made on the fats of invertebrates show no unusual features, apart from those of sponges, which contain a wide range (C_{14}–C_{28}) of unsaturated acids, with the C_{26} and C_{28} constituents predominating (cf. Table V).

Bergmann and Swift (*155*) point out that the high molecular weight acids may be present as cerebroside or phosphatide-like derivatives. However, cerebrosides are not characteristic of the lower forms of life, and as a rule the phospholipid fatty acids of aquatic species are similar to the glyceride fatty acids. In this connection the phospholipids of the mussel (*Mytilus edulis*) have also been found to contain about 10% of higher molecular weight (ca. C_{28}) fatty acids (*155*).

3. *Fats of Marine Fishes and Mammals*

In the elasmobranchs the oil reserves are usually concentrated in liver and the fatty acid composition of the liver oils conforms generally

TABLE VI

The Fatty Acid Composition (Weight %) of Elasmobranch Liver Oils

Species	% Unsaponifiable in oil	Saturated					Unsaturated					
		C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
Skate												
Raia maculata (157)	0.3	4.0	14.0	—	—	—	Trace	10.5 (2.0 H)	20.5 (3.3 H)	32.5 (7.3 H)	18.5 (9.5 H)	—
Shark												
Scymnorhinus lichia (158)	70–80	1.2	14.6	3.6	0.8	0.1	0.4	3.7 (2.0 H)	29.1 (2.0 H)	10.6 (2.0 H)	25.9 (2.1 H)	10.0 (2.0 H)
Galeocerdo rayneri (159), Bay of Bengal	2	1.5	23.6	14.5	0.3	—	0.2 (2.0 H)	10.9 (2.0 H)	23.3 (2.6 H)	11.6 (5.8 H)	12.3 (8.4 H)	1.9 (11.0 H)
Galeocerdo rayneri (159), Arabian Sea	1.5	3.7	24.9	11.1	1.2	—	1.2 (2.0 H)	11.2 (2.6 H)	19.6 (3.9 H)	22.3 (7.0 H)	4.8 (10.6 H)	— —

to that of the average marine type. Within the class, however, several groups have been differentiated [cf. Tsujimoto (*156*)] which, on the basis of their fatty acid compositions, may be described as follows: (*a*) those with large proportions of highly unsaturated fatty acids and low unsaponifiable content (1–2%, mainly sterols); (*b*) those with fatty acids, mainly belonging to the monoethenoid series and containing 10% or more of unsaponifiable matter, which may consist of cholesterol and glyceryl ethers, known as selachyl, chimyl, and batyl alcohols, or of the terpenoid hydrocarbon squalene, in proportions up to 80% of liver oil; (*c*) those with fatty acids containing a high proportion, up to 50%, of saturated fatty acids, and with relatively low proportions of unsaponifiable matter.

The classification outlined is evidently not a rigid one, as some shark (*Galeorhinus* sp.) liver oils with moderate amounts (3–8%) of unsaponifiable matter conform both in regard to fatty acid composition and mean unsaturation of the C_{20} and C_{22} unsaturated acids to the average marine type (*160, 161*). Furthermore, the fatty acid composition of the liver oil is not necessarily determined by biological origin, as marked differences are shown between the same species taken from different waters (see Table VI). On the other hand the data reviewed by Pathak and Dey (*162*) suggest that different species of shark taken off the coast of India are characterized by the high content of saturated acids, as in Tsujimoto's classification (*c*) above. However, the difference in fatty acid composition between the fetal liver fat of *Galcocerdo tigrinus* (with a low content of C_{20} and absence of C_{22} unsaturated acids) and that of the liver fat of the mother, which is of the normal marine type, remains unexplained.

Among the teleostids the most notable exception is the castor oil fish (*Ruvettus pretiosus*), the body fat of which is actually a liquid wax consisting of cetyl, oleyl, and octadecyl alcohols combined with oleic and dihydroxyoleic acids (*163*).

In teleostids, variations in fatty acid composition according to the site of storage have been noted. Hilditch (*3*) observed that on the whole the composition of the flesh fats is more regular and the difference between species less pronounced than in the liver fats. The major component acids in most cases fall within the following limits, expressed as weight per cent of the total fatty acids: palmitic acid, 15–18; unsaturated C_{16}, 10; C_{18}, 20–25; C_{20}, 22–26; and C_{22}, 18–22. Thus the percentages of hexadecenoic, C_{18}, and C_{20} unsaturated acids are somewhat lower, and those of the unsaturated C_{22} acids somewhat higher, than in the typical marine fat.

In teleostids the fat reserves may be concentrated in the liver, as in elasmobranchs, or more or less evenly distributed throughout the tissues.

The liver oils of fishes exhibiting a diffuse oil distribution have been shown by Rapson and co-workers (*164*, *165*) to possess lower iodine values and smaller amounts of C_{20} and C_{22} unsaturated acids than the corresponding liver oils. An extreme example of this phenomenon is that of the New Zealand groper liver oil (*166*). Here the head oils exhibit the usual marine type of fatty acid distribution, while the liver oils contain high proportions of C_{16} and C_{18} unsaturated acids together with abnormally small amounts (7–9%) of C_{20} and especially of C_{22} unsaturated acids, which range from 0 to 4%.

Although the fats of marine fishes taken from different parts of the world generally conform to the average marine type, the limited data on the liver oils taken from fishes of the Southern Hemisphere suggest an enhanced content of C_{18} unsaturated acids together with diminished amounts of C_{20} and C_{22} unsaturated acids [cf. Hilditch (*3*)].

The fairly extensive data on the fats of marine mammals, including whales and seals, show that the fatty acid composition is generally similar to that of marine fishes, but the content of C_{18} unsaturated acids is somewhat higher, and that of the C_{20} and C_{22} unsaturated acids lower. This may be the result of the formation of appreciable amounts of endogenous fat.

The oils from the blubber and head cavity of the sperm whale differ from those of other whales in that they consist mainly of esters of higher aliphatic alcohols. Moreover, the average molecular weight of the fatty acids, which are largely monoethenoid, is lower (*167*).

TABLE VII

FATTY ACID COMPOSITION (WEIGHT %) OF THE BODY BLUBBER OF THE PORPOISE (*168*)

Source	Saturated				Unsaturated				
	C_5	C_{12}	C_{14}	C_{16}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Adult	13.6	3.5	12.1	4.7	4.7	27.2	16.7 (2.8 H)	10.5 (4.8 H)	7.0 (4.9 H)
Fetus	1.2	—	14.9	0.6	12.3	48.1	15.4 (4.0 H)	7.5 (7.4 H)	

The head, jaw, and body oils of dolphins and porpoises contain considerable proportions (up to 25%) of isovaleric acid, which are absent from the organ and liver fats. The fetal blubber fat, in which isovaleric acid is present only in small amounts (1.2%), contains much less C_{20} and C_{22} unsaturated acids than the blubber of the adult (*168*), as shown in Table VII.

4. *Fats of Freshwater Fishes*

The fats of freshwater species generally conform to the average freshwater type described by Lovern (*169*). The flesh fats of the sea trout and the brown trout, which we now regard as migratory and nonmigratory forms of the same species (*Salmo trutta*), show, respectively, the marine and freshwater types of fatty acid composition. Although the sturgeon (*Acipenser sturio*) fats examined by Lovern (*170*) were from a North Sea specimen, it was believed that the freshwater type of fatty acid composition found was associated with feeding in fresh water. Most of the fats of freshwater species examined have been taken from the body, and the concept of an average freshwater type is based on British species. Pathak and Ojha (*171*) found that the body fats of Indian freshwater species were high in C_{18} unsaturated acids but low in C_{20} and C_{22} unsaturated acids, in accordance with the observations made on specimens taken in Great Britain. However, as compared with Lovern's average freshwater type, the Indian species have a much higher content (27–44%) of saturated acids, chiefly palmitic acid, but a lower content of hexadecenoic acid. These investigators suggest that biohydrogenation may occur to a greater degree in the fats of Indian freshwater fishes from tropical waters than in the colder waters of Britain. It was also shown that visceral oils, as compared with the body oils, contained lower proportions of C_{22} unsaturated acids. On the other hand, the liver oil from an Indian species of freshwater fish (*Wallago attu*) examined by Pathak and Agarwal (*172*) showed 19.1% C_{22} and was generally similar in composition to that of marine species.

Of the warm-blooded animals inhabiting fresh water, Meara and Weerakoon (*173*) found that the depot fats of the duckbill platypus (*Ornithorhynchus anatinus*)—an egg-laying mammal—possess the freshwater type of fat.

C. FATS OF TERRESTRIAL ANIMALS

Although only a small part of the total number of species has been examined, particularly among the invertebrates, the data show that the terrestrial animals have depot fats distinct from those of aquatic animals in their relative lack of C_{20} and C_{22} highly unsaturated acids. The depot fats of amphibia and of reptiles are intermediate in this respect between those of more highly evolved land animals and those of aquatic animals. However, this situation may arise from differences in the dietary fat rather than from inherent differences between species.

1. *Fats of Invertebrates*

Hilditch (*3*) refers to the fatty acid composition of the depot fats of six insects of the Orthoptera, the chrysalis oil of the silkworm and the tent moth, as well as the larva fats of a few other groups. The composition of insect fats is variable. The saturated acids comprise 15–35%, with palmitic acid as the main component. In accord perhaps with the green-leaf diet, the unsaturated acids are mainly C_{18} with linoleic and linolenic acids often predominating. C_{20} and C_{22} unsaturated acids appear to be generally absent but have been reported in substantial amounts (26–44%) in the depot fats of two Mexican species (*174*, *175*) including the Mexican locust. The fatty acids from the latter, however, failed to give the polybromide test for C_{20} and C_{22} highly unsaturated acids. The fatty acids from the other Mexican insect gave ether-insoluble bromides which darkened at 200°. The evidence for the presence of polyunsaturated C_{20} and C_{22} acids in these insects is thus rather inconclusive. The effect of the dietary fat is evident on comparing the larva fats of the beetle (*Pachymerus dactris*) with those of the nut (*Manicaria saccifera*) on which the beetles feed (*176*). The behavior of these larvae is similar to that of rats (see Table II) in which dietary myristic, as compared with lauric, acid is preferentially deposited.

Of the remaining land invertebrates may be mentioned the earthworm (Lumbricidae), which contains 1.2–1.3% of lipids, half of which is unsaponifiable. The fatty acids are a third phosphatidic and form an unusual range with C_{10} to C_{18} saturated and C_{12} to above C_{22} unsaturated (*177*).

2. *Fats of Amphibians and Reptiles*

The fatty acid composition of the depot fats of amphibians and of reptiles forms a link between those of aquatic species and terrestrial mammals. The extensive data reviewed by Hilditch (*3*) show that among the amphibia and reptiles there is a wide range of variation, from the depot fat of the frog with 15% C_{20}–C_{22} unsaturated acids and 11% palmitic acid to that of the crocodile with 27% palmitic acid and only 4% C_{20}–C_{22} acids, corresponding closely to the proportions found in the depot fats of mammals. These variations are, perhaps, more closely related to the diet than to species, since Pathak and Pande (*178*) found 12% of C_{20}–C_{22} unsaturated acids in the fats of the crocodile living on its natural diet of fish, as compared with 3.8% found by Gunstone and Russell (*143*) in the fats of a captive crocodile fed on piglets.

Similarly, Hilditch (*3*) records 1.3–14.8% C_{20}–C_{22} acids in the depot fatty acids of different species of turtles, some of which may have been held in captivity for a long time. On the other hand, the depot fatty acids of a marine specimen taken in its natural environment was shown by Pathak and Dey (*141*) to have about 30% of C_{20}–C_{24} unsaturated acids, and, in addition, to resemble quite closely a marine type of fat.

3. *Bird Depot Fats*

It would appear that the depot fats of land birds, of which only five species have been examined, are similar to those of nonruminants. However, whereas the depot fats in different parts of the same bird (*179, 180*) are uniform in composition, differences are found in stearic and C_{18} unsaturated acid content between the different depot fats of the same animal in some mammalian species, such as the pig (cf. Section VI). Depot fats of the hen are readily changed by feeding unsaturated oils (*181*), and the depot fats of the ostrich, fed on maize, reflect the fats of the diet in their high content (17.1%) of C_{18} dienoic acid (*144*). The deposition of the marine type of fat by marine birds (*182*) is further evidence of the influence of the dietary fat.

4. *The Fats of Land Mammals*

The evidence based on the study of relatively few species suggests that the depot fats of ruminants are not appreciably affected by the dietary fat. On the other hand, the depot fats of nonruminants reflect those of the diet. It is therefore convenient to consider the depot fats of ruminants and nonruminants separately.

a. Fats of Nonruminants. The herbivorous animals in this group possess depot fats generally similar to those of the rat, apart from the elephant, whose depot fat has 44.1% palmitic acid [cf. Hilditch (*3*)]. Variations in the amount of dienoic and trienoic acids are probably due to the influence of the dietary fat. For example, the depot fats of grass-fed horses contain 17% linolenic and 4% linoleic (*183*), as compared with 2% linolenic and 22% linoleic acids found in the depot fats of horses fed on oats. These variations accord with the fact that grass fats consist predominantly of linolenic acid, whereas those of oats are rich in linoleic acid (*184*).

Hilditch (*3*) has described the fats of seven species of Carnivora and Omnivora. The data show a content of palmitic acid varying from 18.9 to 29.8%, and of stearic acid from 5.8 to 26.9%.

The unsaturated acids are mainly oleic acid with variable amounts of linoleic and linolenic acids. The fat of the badger, however, is exceptional in containing 14.6% C_{20}–C_{22} unsaturated acids (*185*).

The marked influence of the dietary fat is again suggested by the fact that whereas captive tigers and pumas fed on cow and horse flesh deposit considerable proportions (12–13%) of C_{18} polyene acids derived from horse flesh (see Table VIII), the same animals in their natural habitat, living on sheep and deer with fats of relatively high stearic acid content, deposit these fats more or less unchanged (*149*). Pathak and Trivedi (*186*) have similarly noted a close resemblance in composition between the depot fats of the lion, tiger, and panther and those of the animals on which they feed, including deer, sheep, and cattle. However, in the lion and panther the proportions of tetra- and hexadecenoic acids are somewhat higher, and in addition, the panther fat contains 8.3% of C_{20}–C_{22} unsaturated acids. It is of incidental interest that the glycerides of the panther depot fat followed Hilditch's (*3*) even-distribution rule in contrast to the glycerides of the dietary fat which contain a much higher content of fully saturated glycerides than that calculated from this rule. Among the fats of Omnivora may be included those of humans, which are doubtless influenced by the diet. The human depot fats examined by Cramer and Brown (*187*), apart from the presence of more (8.2–10.2%) octadecadienoic acid, corresponded closely to those of rats fed a diet low in fat. The detailed study of the fatty acid composition of rodent depot fats is confined to one species—the rat. Details of the composition of the depot fat of this species and the influence of the dietary fat on its composition have been earlier described (see Table II). The fatty acid composition of the depot fats of the mouse and the porcupine are also shown in Table III. Both the mouse (*146*) and the rat (*81*) are shown to have similar proportions of palmitic acid, 25–30%, to those found in other mammalian depot fats, but tend to have somewhat lower proportions of stearic acid. The porcupine (*146*), on the other hand, has a relatively high content of palmitic acid amounting to 36.3%.

b. Ruminant Depot Fats. The evidence so far indicates the marked influence of the dietary fat on the composition of the depot fats of nonruminants (cf. also Table II). In contrast Shorland (*188*) has shown that the fats of ruminants are not markedly affected by the dietary fats and that the unsaturated acids of the diet are wholly or partially hydrogenated by the rumen microorganisms, leading to the formation of depot fats which consist largely of palmitic acid, 25–30%, stearic acid, 10–30%, and oleic acid or isomers together with small amounts of other mono-, di-, and polyunsaturated acids (*189*). The specific occurrence of *trans* unsaturated acids in ruminants, as earlier discussed, is related to the hydrogenating mechanisms of the microorganisms in the rumen (cf. Table VIII). The traces of *n*-odd-numbered

TABLE VIII

COMPARISON BETWEEN THE FATTY ACID COMPOSITION (WEIGHT %) OF THE DIETARY FATS AND THE DEPOT FATS OF PASTURE-FED ANIMALS *(89)*

Species	Saturated					Unsaturated							
	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{12}	C_{14}	C_{16}	C_{18}			C_{20}–C_{22}	*Trans* acids *(121)*
									Monoene	Diene	Triene		
Ryegrass (leaf glycerides)[a]	0.4	1.4	10.6	1.5	0.4	0.2	0.5	4.1	4.6	11.6	62.8	1.9	Trace
Nonruminants													
Wild rabbit (abdominal)	—	1.6	22.1	6.4	0.8	—	0.4	4.4	12.7	7.9	42.4	1.3	Not detected
Horse (total fatty tissues)	—	2.4	29.7	4.3	0.2	—	1.4	6.5	32.5	3.8	16.1	3.1	Not detected
Ruminants													
Deer (perinephric)	0.1	5.1	35.9	29.6	2.9	—	0.2	2.2	17.0	1.2	1.0	4.8	3.5
Ox (caul and kidney)	—	2.7	27.8	21.6	—	—	0.3	2.5	42.5	0.5	0.3	1.8	4.5
Sheep (total fatty tissues)	1.3	3.5	25.0	22.2	0.7	—	0.5	1.7	44.2	Trace	Trace	0.9	11.2

[a] See also Section IV,*D*,*2*,*a*.

acids as well as even- and odd-numbered iso and odd-numbered anteiso acids found in the rumen similarly result from the lower homologs produced by degradation of the proteins and carbohydrates in the rumen [cf. Shorland (*189*)].

The fatty acid composition of the organ fats of animals as well as the milk fats and fats secreted by sebaceous glands is discussed in Section VI.

D. PLANT FATS

Included in this group are the fats of the simpler forms of plant life, other than those already referred to in connection with aquatic fats. In some of the cryptogams, and especially in bacteria, the lipids are sometimes very complex and triglycerides are either absent or form a minor part of the total lipid. Nevertheless, it is convenient to discuss their fatty acids alongside those of the higher plants.

In the phanerogams, the seed fats and, to a lesser extent, the fruit-coat fats have been investigated. There is also information about the composition of leaf lipids, but the lipids in other parts of the plants have not yet been closely studied.

1. *Cryptogam Fats*

The component fatty acids of cryptogam fats have been given only limited attention, and it is likely that further investigation will alter current conceptions regarding the kinds of lipids and fatty acid types in this group. Nevertheless, as will be shown in the description that follows, many features not found in phanerogam lipids will be revealed, as will the surprising differences between the species belonging to the same family.

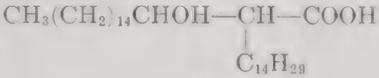
a. Fats of Bacteria. Among the bacterial lipids, the acid-fast bacteria, including especially tubercle bacilli, have received much attention.

The lipids of the acid-fast bacteria are unusual in lacking any appreciable amounts of the glycerides that form the most important part of the depot fats of other species. The main constituents of acid-fast bacteria are waxes of higher molecular weight branched-chain acids (mycolic acids) and polysaccharides (*190, 191*).

The acetone-soluble "fats" of the acid-fast bacteria examined contain in addition to palmitic, stearic, oleic and linoleic acids, characteristic branched-chain fatty acids. Similar acids are also found in the phospholipids. For example, in *Mycobacterium leprae* "fat" there are present, dextrorotatory branched-chain acids with 16, 19, and 22 carbon atoms (*192*), whereas human tubercle bacilli "fat" contains tuberculostearic (D(—)-10-methyl stearic) acid (*51*, *193*).

The higher molecular weight phthioic ($C_{26}H_{52}O_2$) acid, with three branched methyl groups, isolated by Anderson (*194*) has since proved to be a mixture from which several components have been separated. These include (+)-2,4,6-trimethyltetracos-2-enoic (mycolipenic) acid (*52*) and (—)-2,4,6-trimethyloctacosanoic (mycoceranic) acid (*53*). Thus the same organism is shown to elaborate acids with similar structures but of opposite configuration.

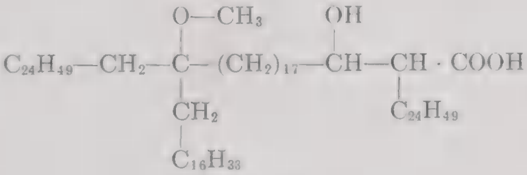
The waxes of the acid-fast bacteria are quantitatively the most important part of the lipid fraction and contain, as the fatty acid constituents, mycolic acids (*195*). The simplest member of this series occurs in *Corynebacterium diphtheriae* (*196*) and has the structure shown (XII).



$$CH_3(CH_2)_{14}CHOH{-}\underset{\substack{|\\ C_{14}H_{29}}}{CH}{-}COOH$$

(XII)

The typical mycolic acids found in acid-fast bacteria, however, contain about 88 carbon atoms and vary slightly in structure with different organisms (*197*) within this group of bacteria. Detailed studies (*198*) on the α-mycolic acid from the strain "Test" indicate the structure shown (XIII).



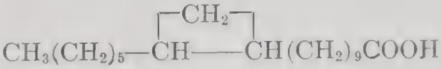
$$C_{24}H_{49}{-}CH_2{-}\overset{\substack{O-CH_3\\ |}}{\underset{\substack{|\\ CH_2\\ |\\ C_{16}H_{33}}}{C}}{-}(CH_2)_{17}{-}\overset{\substack{OH\\ |}}{CH}{-}\underset{\substack{|\\ C_{24}H_{49}}}{CH}\cdot COOH$$

(XIII)

In addition to the branched-chain fatty acids described (+)-6-methyloctanoic acid has been isolated from the antibiotic substances of *Bacillus polymyxa* (*199*, *200*).

The branched-chain hydroxy acids of the mycolic type have been mentioned. In addition, there occur in several species straight-chain hydroxy-substituted acids. In *Bacillus megatherium* there is a lipid which is a polymer consisting of the etholide of β-hydroxybutyric acid (*201*, *202*). This acid is released on hydrolysis: D(—)-β-hydroxydecanoic acid occurs in *Pseudomonas pyocyanea* (*203*), D(—)-β-hydroxymyristic acid in *Escherichia coli* (*204*), and dihydroxystearic acid in *Lactobacillus acidophilus* (*205*).

Of the other groups of bacteria which have received detailed attention may be mentioned the lactobacilli. Those examined by Hofmann and co-workers include *L. arabinosis*, *L. casei*, and *L. delbrueckii*. Their lipids contain, in addition to the series of *n*-saturated (mainly palmitic) acids, octadec-*cis*-11-enoic, and lactobacillic acids (XIV).



$$CH_3(CH_2)_5\text{—}\overset{\ulcorner\text{—}CH_2\text{—}\urcorner}{CH\text{——}CH}(CH_2)_9COOH$$

(XIV)

The last two acids are also main constituents of *Phytomonas tumefaciens*. On the other hand, the *Streptococcus* species has *cis*-vaccenic but no lactobacillic acid (*206*).

The bacterial lipids have been studied in a somewhat fragmentary manner, but the lipids have been shown to vary greatly in the different species. The complex lipids present in tubercle bacilli contain unique types of branched-chain fatty acids whereas the lactobacilli are unusual in elaborating lactobacillic and *cis*-vaccenic acids. Apart from *cis*-vaccenic acid the fatty acids of *Streptococcus* species show no unusual features (*207*). The absence of sterols distinguishes bacteria from other forms of life (*198*).

The pattern of fatty acid occurrence in bacterial lipids thus shows a uniqueness, and at the same time a diversity between different groups of bacteria.

TABLE IX

The Component Fatty Acids (Weight %) of Yeast Fats

Component	*Torulopsis utilis* (*208*)	Yeast strain No. 72 (*209*)	*Rhodotorula gracilis* (*210*)	*Rhodotorula graminis* (*211*)
Total lipids (gm./100 gm. dry weight)	6.4	25–33	49.8	37.2
Steam volatile acids	4.6	—	—	0.8
Myristic	0.3	0.1	1.1	3.9
Palmitic	7.9	25.6	29.8	31.9
Stearic	3.8	5.9	8.8	3.2
Saturated C_{20} and above	0.2	5.1	1.4	—
Tetradecenoic	—	—	—	1.1
Hexadecenoic	7.6	1.3	1.8	0.3
Oleic	21.5	54.5	40.1	37.2
Linoleic	49.7	5.7	11.2	10.2
Linolenic	4.4	0.7	4.8	4.6
Unsaturated C_{20}	—	1.1	1.0	2.5[a]

[a] Also 4.3% of unidentified acids, of which 3.9% was saturated.

TABLE X

THE FATTY ACID COMPOSITION (WEIGHT %) OF MOLD FATS

				Penicillium					
	Aspergillus nidulans (*212*)	*Phycomyces blakesleeanus* (*213*)	*Neurospora crassa* (*214*)	*P. javanicum* (*215*)	*P.* sp. (*216*)	*P. flavo-cinereum* (*217*)	*P. lilacinum* (*218*)	Peanut oil (*219*)	Pig fat (*147*)
Myristic	0.7	—	15	—	3.5	0.3	0.1	0.4	1.3
Palmitic	20.9	23.7		23.4	40.5	19.4	32.3	9.4	28.3
Stearic	15.9	4.7		9.4	7.9	9.9	9.4	3.1	11.9
Saturated C_{20} and above	1.4	5.0		0.8	1.1	0.7	1.4	5.1	0.2
Hexadecenoic	1.2	—	2[b]	—	7.9	1.5	3.4	0.9	2.7
Oleic	40.3	29.6	5	34.6	19.0	39.4	38.6	54.9	47.5
Linoleic	17.0	25.8	43	31.8	20.1	27.1	13.4	26.2	6.0
Linolenic	0.2	—	32	—	—	0.8	—	—	—
C_{20}–C_{22}	2.4	—[a]	2	—	—	0.9	1.4	—	2.1

[a] Also octadeca-6,9,12-trienoic acid, 3.4%; tetracos-17-enoic acid, 2.9%; and a hexacosenoic acid, 4.9%.

[b] Also 1% hexadecadienoic acid.

b. Yeast and Mold Fats. It is well known that yeasts and molds when cultured on suitable media will yield a high percentage of fat. The fatty acids appear to be generally similar to those found in the seed oils of many plants, the main constituents being oleic and linoleic acids, together with lesser amounts of palmitic and stearic acids (see Tables IX and X). The molds and yeasts are shown to elaborate similar fats, the palmitic acid content in many instances approaching or even exceeding the 25–30% characteristic of the depot fats of higher animals. The stearic acid content is usually below 10%, but rises in *Aspergillus nidulans* to 15.9%, which approaches the amount usually found in ruminant fats. The content of linoleic acid is variable, covering a wide range from 5.7 to 49.7%. Considered collectively, the results show that the fats of molds and yeasts contain no characteristic properties. The most abundant acids—oleic, linoleic, palmitic, and stearic—are those most commonly found in plant fats. Linolenic acid is usually present in trace amounts, but in *Neurospora crassa* this acid amounts to 32% of the total fatty acids (*214*). It is perhaps not surprising, in view of the variability in the proportions of these commonly occurring fatty acids, that fats of some yeasts or molds should correspond closely to those found in higher plants and animals. Thus the fats of *Penicillium lilacinum* and of pig fat are of similar fatty acid composition, and the composition of the linoleic-rich molds and yeasts is not unlike that of cottonseed oil. It is therefore not feasible from the types and distribution of the fatty acids to deduce whether a given fat has come from a yeast or a mold on the one hand, or from a plant or a pig on the other. It is thus not always possible to correlate fatty acid composition and the classification of species. Nevertheless, within the molds some specificity in fatty acid composition is found. *Phycomyces blakesleeanus* elaborates an unusual isomer of linolenic acid together with tetracos-17-enoic and hexacosenoic acids.

In the uredospore stage of the fungus *Puccinia graminis* var. *tritici*, the glycerides contain as the main constituent palmitic acid (47.4%) with minor amounts of linoleic (3.3%) and linolenic (6.3% acids) (*219a*), the only other important acid being the unique *cis*-9,10-epoxyoctadecanoic (up to 27%) along with (+)-threo-9,10-dihydroxyoctadecanoic acid (up to 5%) (*219b*). The last mentioned acid also occurs in the oil of *Lycopodium clavatum* spores belonging to the order Lycopodiales (*219c*). The occurrence of other epoxy acids in seed fats will be referred to later.

2. *Phanerogam Fats*

As previously mentioned, fats other than seed, fruit-coat, and leaf fats have not been investigated sufficiently to establish any useful relationships. Bark, stem, root, and flower fats have not been found to contain any but the common acids, apart from erucic acid which has been found in the roots of the mangel (*Beta rapa vulgaris*) (*220*).

It is not feasible here to provide details of the extensive data on phanerogam fats, for which the reader is referred to the comprehensive and complete treatise by Hilditch (*3*). The data regarding the seed fats in particular are now voluminous, and in this section it has been necessary to select examples, rather than cover the whole field. In the grouping of seed fats according to the main fatty acid components, the writer has followed essentially the classification described by Hilditch (*3*).

a. Leaf Fats. The leaf fats of only a few species, including especially pasture plants, have been studied. It appears that the lipid content does not usually exceed 7% and that rather less than half of the total lipids are triglycerides (*221*). The remainder comprises waxes, phospholipids, and unsaponifiable matter. The analysis of the glycerides of ryegrass (*Lolium perenne*) shown in Table VIII may be used to illustrate the fatty acid composition of leaf fats.

The above analysis is supported by that of Hilditch and Jasperson (*222*) for grass fatty acids, using similar alkali-isomerization techniques in association with ester fractionation. Earlier work on the leaf glycerides of orchardgrass (cocksfoot) (*Dactylis glomerata*) (*223, 224*) and ryegrass (*223*), as well as on mixed pasture species (*225*), also shows a similar fatty acid composition; but the rather lower proportions of linolenic acid reported are perhaps to be ascribed to the use of the Kaufmann thiocyanogen method in place of the more recent spectroscopic techniques.

The fatty acid composition of the leaf fats of the Cruciferae appears to be similar to that of the Gramineae as shown by the results obtained by Chibnall and Channon (*226*) for cabbage (*Brassica oleracea*) leaf and for rape (*Brassica napus*) leaf by Shorland (*32, 227*). However, in the rape leaf, in addition to a high content of highly unsaturated C_{18} acids, there were also substantial amounts (11–17%) of C_{16} unsaturated acids consisting mainly of hexadeca-7,10,13-trienoic acid which could not be found in ryegrass and appears to be characteristic of rape leaf. Other leaf fats examined, including those of spinach (*Spinacea oleracea*) (*228*), lucerne (*Medicago sativa*) (*229*), and buckwheat (*Polygonum fagopyrum*) (*230*), also show that linolenic acid is the main constituent, though in these species oleic acid is a more important constituent than in the grasses. It is perhaps relevant that oleic acid itself has not been isolated from grass lipids, and the only evidence, apart from physical measurements, on the mixed acids is the isolation of traces of the characteristic dihydroxy acid following alkaline permanganate oxidation (*224*).

The most substantial deviation in the fatty acid composition of leaf material is to be found in nettle (*Urtica dioica*) leaf fat which has mainly oleic acid, 82–86% with 13–14% linoleic acid, 0.5% saturated acids, and, perhaps, some linolenic acid (*231*).

As the limited data indicate, leaf fats are predominantly rich in linolenic and linoleic (or isomers) acids, with oleic acid usually a minor constituent.

Recent work has established that what has been regarded as the triglyceride fraction of leaves is in fact of a much more complex nature. The acetone-soluble lipids of leaves seem to be glycosides containing varying amounts of galactose and resembling those found earlier by Carter *et al.* (*231a*) in wheat flour. The acetone-soluble lipids of the leaves of red clover (*Trifolium pratense*) (*231b,c*) and of ryegrass (*231d*) have been shown to consist of the linolenates of galactosyl-1-glycerol and of digalactosyl-1-glycerol. The linolenic acid is attached to the glycerol radicle. Similar galactolipids have been reported in the chloroplasts of the leaves of the spinach (*Spinacea oleracea*) (*231e*) and in chlorella (*231f*).

b. Fruit-Coat Fats. The fleshy or succulent parts of fruits in some instances contain considerable proportions of fat, the most familiar examples being olive oil and red palm oil. Hilditch (*3*) lists the fatty acid composition of the fruit-coat fats of fifteen families, showing that palmitic, oleic, and linoleic acids are the main constituents. The differentiation between the families is one of variation in the proportions of oleic, palmitic and linoleic acids, rather than in the occurrence of specific fatty acids. However, certain members of the laurel family have been found to yield fruit-coat fats with up to 33% lauric acid (*232*). These variations have been briefly summarized by Hilditch (*233*), as shown in Table XI.

TABLE XI
COMPONENT ACIDS OF FRUIT-COAT FATS

Species	Fatty acids (wt. %) (*233*)		
	Palmitic	Oleic	Linoleic
Olea europaea (olive oil)	10	70–80	7–10
Lauris nobilis (laurel oil)	20	63	14
Elaeis guineensis (palm oil)	35–43	40–50	7–10
Stillingia sebifera (stillingia tallow)	66	30	—
Rhus sp. (sumach tallow)	75	13	—

The fruit-coat fats vary in consistency from oils liquid at ordinary temperature, such as olive oil, to hard waxlike materials, such as stillingia and sumach tallows, which do not melt below about 50°.

c. Seed Fats. The classification of the seed fats on the basis of their chief component fatty acids, as outlined by Hilditch (*3*), is followed in this section. This leads in many instances to the grouping together of members of the same family. Broadly, fats which contain the typical acids, viz., oleic, palmitic, linoleic, and linolenic acids are first considered and arranged in descending order of mean unsaturation; this gives groups corresponding to drying, semidrying, and nondrying oils. Subsequently, fats containing a characteristic fatty acid, other than those mentioned above, are grouped together under the fatty acid concerned. In this system it is convenient to list the names of families which include species whose fats fall into line with the fatty acids upon which the classification is based. This does not, however, necessarily imply that all the species within the families mentioned fall into the classification thus arranged. In some cases the same family will appear under two different classifications as members within the family fall into separate categories. Thus the *Ulmaceae* family appears under the linoleic acid-rich fats as well as under fats which contain *n*-decanoic (capric) acid as the main constituent.

(*1*) *Seed fats with linoleic (oleic) and linolenic acids as major components; minor components, palmitic and stearic acids.*

(*a*) Linolenic acid-rich seed fats: In this group the following families are represented: Gymnosperms—Coniferae; Angiosperms—Maracaceae, Celastraceae, Aquifoliaceae, Labiatae, Valerianaceae, Oenotheraceae, Linaceae, Elaeagnaceae, and Rhamnaceae.

The best-known example of the linolenic acid-rich group is linseed (*Linum usitatissimum*) oil (Linaceae), which contains saturated (mainly palmitic) 6–16%, oleic 13–36%, linoleic 10–25%, and linolenic 30–60%, acids. In the various families with linolenic acid-rich seed oils, the linolenic acid content does not usually exceed 50% except in the Labiatae. A notable example here is perilla (*Perilla ocimoides*) seed oil with up to 70% linolenic acid. Although the seed fats of the families listed are essentially composed of palmitic, oleic, linoleic, and linolenic acids, in the seed oil of the evening primrose (*Oenothera biennis*), a species of Oenotheraceae, linolenic (octadeca-9,12,15-trienoic) acid itself is absent. However, an isomer of linolenic acid, octadeca-6,9,12-trienoic acid, occurs (*234, 235*).

(*b*) Mainly linoleic acid-rich seed fats: In this group the saturated acids, mainly palmitic, form 4–20% of the total fatty acids. Linolenic acid is present in small amounts only, linoleic acid frequently forms more than 50%, but sometimes oleic acid predominates.

Botanical families represented include Betulaceae, Fagaceae, Juglandaceae, Hippocastanaceae, Ulmaceae, Olacaceae, Vitaceae, Papaveraceae, Passifloraceae, Typhaceae, Compositae, Solanaceae, Pedaliaceae, Scrophulariaciae, Plantaginaceae, Dipsaceae, Asclepiadaceae, Staphyleaceae, Myrtaceae, Urticaceae, Theaceae, Oleaceae, and Proteaceae.

Some well-known examples of this group are collected together in Table XII.

TABLE XII
LINOLEIC ACID-RICH SEED OILS

Family	Species	Fatty acids (wt. %)				
		Palmitic	Stearic	Oleic	Linoleic	Linolenic
Juglandaceae	Walnut (*Juglans regia*) (*236*)	7.0	1.0	16	72	4
Papaveraceae	Poppy (*Papaver somniferum*) (*237*)	8.3	2.7	16.4	72.6	—
Pedaliaceae	Sesame (*Sesamum indicum*) (*238*)	8.2	3.6[a]	45.3	41.2	—

[a] Also 1.1% arachidic and 0.5% hexadecenoic acid.

In a few members of the above families there have been reported substantial amounts of fatty acids other than linoleic, oleic, palmitic, and stearic. In ivory wood (*Agonandra brasiliensis*) seed oil (Olacaceae) is found 50% ricinoleic (12-hydroxyoctadec-9-enoic) acid (*239*). Vernolic (12,13-epoxyoctadec-9-enoic) acid is a major fatty acid in *Vernonia anthelmintica* oil (Compositae) (*26*), 20% palmitoleic acid is found in the seed oil of *Macadamia ternifolia* (Proteaceae) (*240*), and higher unsaturated acids including hexacos-17-enoic and tricos-21-enoic acids in the seed oils of *Ximenia americana* (Olacaceae) (*241*).

(2) *Seed fats with linoleic, oleic, linolenic, or a conjugated polyethenoid acid (eleostearic, licanic, etc.) as major component acids. Minor component acids: palmitic (stearic).* Included in this group are the families Rosaceae, Euphorbiaceae, and Curcubitaceae. Some species of these families elaborate seed fats as in groups described in *1a* and *1b* above. In the Rosaceae many of the species elaborate in their seed fats α-eleostearic (octadeca-*cis*-9,*trans*-11,*trans*-13-trienoic) acid or licanic (4-ketooctadeca-*cis*-9,*trans*-11,*trans*-13-trienoic) acid or both. In addition, parinaric (octadeca-9,11,13,15-tetraenoic) acid may occur. The composition of some Rosaceae seed oils is shown in Table XIII.

In the Euphorbiaceae, the seeds of some species, such as Japanese tung (*Aleurites cordata*), elaborate oils rich in eleostearic acid. In the seed oils of other species, such as conophor (*Tetracarpidium conophorum* Awusa), which contains much (63–68%) linolenic acid, there are no unusual acids (*244*). Castor (*Ricinus communis*) seed oil stands apart with its high content (approximately 90%) of ricinoleic acid (*3*).

TABLE XIII

COMPONENT FATTY ACIDS (WEIGHT %) OF ROSACEAE SEED FATS

Species	Palmitic	Stearic	Oleic	Linoleic	Polyenoic
Licania rigida (*242*)	←——11——→		6	5	61 (licanic), 17 (eleostearic)
Parinarium laurinum (*243*)	4	1	10	4	51 (parinaric), 30 (eleostearic)

The same acid was believed to occur to the extent of 33% in the seed oil of *Cephalocroton cordofanus*, which also belongs to the same family. However, the acid has since been shown to be vernolic (*cis*-12,13-epoxyoctadec-*cis*-9-enoic) acid (*245*).

Other examples of the occurrence of unusual fatty acids in the Euphorbiaceae include that of ω-hydroxyeleostearic acid, the main constituent of kamala (*Mallotus philippinensis*) seed oil (*36*, *246*), deca-2,4-dienoic acid found in minor amounts in Stillingia (*Sapium sebiferum*-chinese tallow tree) seed oils (*29*) and the corresponding dodeca-2,4-dienoic acid which occurs in *Sebastiana ligustrina* (*247*).

The Cucurbitaceae seed fats are mostly linoleic acid-rich fats with considerable proportions of oleic acid. A few species, however, contain in their seed oils, in addition, trichosanic acid, which is now considered to be identical with punicic (octadeca-*cis*-9,*cis*-11,*trans*-13-trienoic) acid (*248*, *249*), the major constituent of pomegranate (*Punica granatum*, family Punicaceae) seed oil. Although the Compositae family have been classified in the linoleic acid-rich group it is appropriate to mention here that one species, Scots marigold (*Calendula officinalis*), contains in its seed oil the unique acid, octadeca-8,10,12-trienoic acid. It is believed to be the all-*trans* isomer and therefore related to β-eleostearic acid (*250*) and classifiable in group (*2*).

(3) *Seed fats containing, as major component acids, palmitic oleic, and linoleic acids.* In this group the following families are represented: Berberidaceae, Magnoliaceae, Anonaceae, Rutaceae, Zygophyllaceae, Anacardiaceae, Capparidaceae, Hernandiaceae, Tiliaceae, Malvaceae, Bombacaceae, Caryocaraceae, Caricaceae, Lecythidaceae, Combretaceae, Apocynaceae, Amarantaceae, Martyniaceae, Acanthaceae, Bignoniaceae, Rubiaceae, and Caprifoliaceae.

Although many seed oils in the group have been examined, their fatty acid compositions usually conform fairly closely to that of cottonseed (*Gossypium arboreum*) oil (*251*), which has the following fatty acid composition (weight %): myristic, 3.3; palmitic, 19.9; stearic, 1.3; arachidic, 0.6; oleic, 29.6; and linoleic, 45.3.

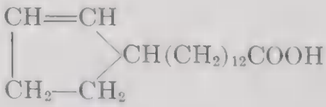
The regular monotony is broken by the appearance of 9-hydroxyoctadec-12-enoic acid in the seed oils of the *Strophanthus* genus (Apocyanaceae) (*25, 252*). Reference has been made to the occurrence of vernolic (12,13-epoxyoctadec-9-enoic) acid in the seed oils of *Vernonia anthelmintica* (Compositae) and in *Cephalocroton cordofanus* (Euphorbiaceae). Recently this acid has also been shown to be present in the seed oil of *Hibiscus esculentus* Linn (*253*). In the same family (Malvaceae) there has also been reported in the seed oil of *Malva verticillata* and *M. parviflora* the occurrence of a C_{18} acid containing a cyclopropene ring analogous in structure to sterculic acid (*254, 255*).

(*4*) *Seed fats containing characteristic acids other than or in addition to oleic, linoleic, and palmitic acids.*

(*a*) Seed fats with petroselinic (octadec-6-enoic) acid as a major component: Petroselinic acid is characteristic of the Umbelliferae family, and is also known to occur in the Araliaceae family. A well known example is parsley (*Petroselinum sativum*) seed oil, which has the following fatty acid composition (weight %): palmitic, 3; oleic, 15; linoleic, 6; and petroselinic, 76 (*256*).

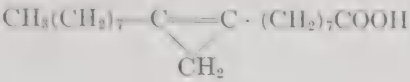
(*b*) Seed fats with tariric or other ethynoid acid as a major component: Tariric (octadec-6-ynoic) acid occurs in the genus *Picramnia.* a member of the Simarubaceae family, the seed fats of other plants belonging to this family being variable and quite different. In *Picramnia* sp. seed fat, Steger and van Loon found 95% tariric acid (*257*) *Onguekoa gore* (Olacaceae) seed fat contains chiefly 8-hydroxyoctadec-17-en-9,11-diynoic (*258*) and octadec-17-en-9,11-diynoic acids (*42*). Another genus of the Olacaceae family, *Ximenia,* contains in its seed fats octadec-11-en-9-ynoic acid (ximenynic acid) (*43, 44*). The 11,12 double bond has the *trans* configuration (*259*). The seed fats of Santalaceae species have also been shown to contain ximenynic acid (*260, 261*).

(*c*) Seed fats with cyclic unsaturated acids as major components: The C_{16} and C_{18} acids with a terminal cyclopentenyl ring, as in chaulmoogric acid (XV)



(XV)

are found in the seed fats of many members of the Flacourtiaceae, but not elsewhere (*3*). The only other known occurrence of cyclic acids in seed fats is that of sterculic acid (XVI)



$$CH_3(CH_2)_7\text{—}\underset{CH_2}{C{=}C}\cdot(CH_2)_7COOH$$

(XVI)

found in *Sterculia foetida* (*55*) and the related C_{18} acid malvalic acid found in *Malva verticillata* and *M. parviflora* (*254*). The seed fats of the families to which these species belong, Sterculiaceae and Malvaceae, respectively, are characterized by the presence of palmitic and stearic acids, the remaining acids being oleic and linoleic [cf. groups (*3*) and (*4h*)].

(*d*) Seed fats with eicos-11-enoic acid as a major constituent: In the seed oils of several species of the Sapindaceae, minor amounts (3–9%) of eicos-11-enoic acid have been reported, but in *Cardiospermum halicacabum* L., this acid forms 42% of the total fatty acids (*262*). Eicos-11-enoic acid is also the major fatty acid constituent of the seed lipids of *Simmondsia californica* belonging to the Buxaceae family (*263*). These lipids are unique in seeds as the glycerides are completely replaced by wax esters of higher unsaturated fatty acids with higher unsaturated alcohols.

(*e*) Seed fats with erucic acid as a major component: Erucic (docos-13-enoic) acid is the main component of the seed fats of the Cruciferae. It has been found also in the *Tropaeolum* seed fats. As an example of Cruciferae seed fats may be mentioned rape (*Brassica campestris*) seed oil, which has the following composition: palmitic, 2; C_{18}–C_{24} saturated, 5.5; hexadecenoic, 1.5; oleic, 12.5; linoleic, 16.0; linolenic, 8.5; eicosenoic, 5; erucic, 47.5; and docosadienoic, 1.5% (*264*).

Hilditch and Meara (*265*) found that the seed fats of *Tropaeolum minus* contain 82.8% erucic, together with 16.0% oleic, 1.2% linoleic, and 1.0% saturated. Associated with the occurrence of erucic acid is eicos-11-enoic acid, mentioned above.

(*f*) Seed fats with unsaturated fatty acids of higher molecular weight than erucic acid: The occurrence in seed fats of unsaturated acids of higher molecular weight than erucic acid is rare. However, the unusual seed fats of *Ximenia* species (Olacaceae) contain small amounts of the monoethenoid acids of the C_{20}, C_{22}, C_{24}, C_{26}, C_{28}, and C_{30} series of the general formula $CH_3(CH_2)_7CH=CH(CH_2)_xCOOH$ (*266*). In addition, small amounts of tetracosenoic and docosenoic acids, along with larger amounts (approximately 10%) of eicosenoic acids, have been reported in the seed fats of *Pongamia glabra* (Leguminosae) (*267*).

(*g*) Seed fats with higher saturated fatty acids: These are represented by the Leguminosae, Moringaceae, Ochnaceae, and Sapindaceae. In some instances, the higher saturated acids may attain considerable proportions as in *Adenanthera pavonina* (Leguminosae) seed fats, which contain 25% lignoceric acid (*268*). The seed oil of *Adhatoda vasica* of the Acanthaceae family is also exceptionally rich in the higher saturated acids and contains 3.1% arachidic, 22.2% behenic, 10.7% lignoceric, and 5.0% cerotic acids (*269*). Other members of this family are classified with the palmitic acid-rich fats. The fatty acid composition of peanut (*Arachis hypogaea*) oil is perhaps typical of this group in containing 5.1–9.9% of C_{20} to C_{24} saturated acids (*3*).

(*h*) Seed fats of which stearic acid is major component: Gnetaceae, Meliaceae, Menispermaceae, Sterculiaceae, Guttiferae, Dipterocarpaceae, Burseraceae, Sapotaceae, Convolvulaceae, and Verbenaceae.

Most seed fats are characterized by a low content of stearic acid. In the families in the stearic acid-rich group, however, the content of stearic acid usually ranges between 15 and 60%. The fatty acid composition (weight %) may be illustrated by that of the seed fat of Kokum butter from *Garcinia indica* (Guttiferae) as follows: stearic, 56.4; palmitic, 2.5; oleic, 39.4; and linoleic, 1.7 (*270*).

(*i*) Seed fats of which myristic and lauric acids are major components: In this group the following botanical families are represented: Lauraceae, Myristicaceae, Simarubaceae, Vochysiaceae, Salvadoraceae, and Palmae. The nutmeg, *Myristica fragrans* (Myristicaceae), seed oil is illustrative of a myristic acid-rich fat. It contains (wt. %) 1.5 lauric, 76.6 myristic, 10.1 palmitic, 10.5 oleic, and 1.3 linoleic acids (*271*). The fatty acid composition (wt. %) of the lauric acid-rich seed fats may be exemplified by that of the palm, *Elaeis guineensis* (Palmae), as follows: octanoic, 2.4; decanoic, 3.7; lauric, 45.2; myristic, 18.6; palmitic, 8.5; stearic, 2.5; arachidic, 1.9; oleic, 15.1; and linoleic, 2.1 (*272*).

(*j*) Seed fats of which capric (decanoic) acid is a major component: This group is represented by the Ulmaceae, in which the seed oils of the eight species of elm, and several varieties of these species so far examined, contain 50–73% decanoic acid [cf. Hilditch (*3*) and Sørensen and Søltoft (*273*)]. Their fatty acid composition (weight %) is represented by that of the American elm (*Ulmus americana*) seed as follows: octanoic, 5.3; decanoic, 61.3; lauric, 5.9; myristic, 4.6; palmitic, 2.9; oleic, 11.0; and linoleic, 9.0. As earlier indicated, certain other species of Ulmaceae seed oils belong to the linoleic-rich group.

(5) *General conclusions regarding the fatty acid occurrence and*

composition of phanerogam fats. In view of the extensive and varied nature of the data discussed in the present section it is perhaps advisable to summarize the main conclusions that emerge.

In the seed fats of many species, as in the leaf and fruit-coat fats, the fatty acids are often limited to the unsaturated acids, oleic and linoleic (sometimes with linolenic acid), and palmitic acid is the chief saturated acid. However, in other species there are found in the seed fats specific fatty acids characteristic of the family or the genus. Thus the occurrence of petroselinic acid is characteristic of the Umbelliferae, chaulmoogric and similar cyclopentenyl fatty acids are found only in the Flacourtiaceae. In the Cruciferae, Hilditch (3) lists the seed fats of five species of *Brassica* and eleven other genera of the family. In all but one species, *Hesperis matronalis*, erucic acid occurs and is usually a major constituent. A high content of myristic acid likewise is found in the seed fats of all species of Myristicaceae, and seed fats of various Palmae show a great similarity in fatty acid composition as well as a high (approximately 45%) lauric acid content. These examples show a striking correlation between the chemical and morphological classification of species. There are, however, other examples where the correlation is more limited or even absent. In the Euphorbiaceae, Hilditch (3) lists the fatty acid composition of 33 species belonging to 21 genera. The polyunsaturated acids, which are typically the main constituents, comprise in some species linolenic acid, in others α-eleostearic acid, or as in *Mallotus philippinensis*, ω-hydroxyeleostearic acid. Again, polyunsaturated acids may be absent and their place be taken by unusual acids such as ricinoleic in castor (*Ricinus communis*) oil, or epoxyoleic acid in *Cephalocroton cordofanus.* However, if the fatty acid composition of individual genera is considered, there is considerable specificity. In the genus *Aleurites*, for example, four different species are listed. In three, the major fatty acid is α-eleostearic. In the other species α-eleostearic acid is absent, being replaced by linolenic acid. In the genus *Euphorbia* the four species listed all contain linolenic acid as the only polyunsaturated constituent. The Simarubaceae include a diversity of seed fats in the different species. Those of the genus *Picramnia* contain tariric acid; those of *Irvingia*, myristic and lauric; those of *Picrasma*, petroselinic acid; and other genera have seed fats that are classifiable into the large category of the palmitic-oleic-linoleic group.

The discovery of a new or unusual fatty acid in a seed fat does not necessarily place the plant in a particular botanical family or signify the genus or species to which it belongs. Vernolic acid, which was earlier considered to be specific for the seed fat of *Vernonia anthel-*

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mintica (Compositae), has now been found in the Euphorbiaceae as well as in the Malvaceae. Similarly, the occurrence of a fatty acid with a cyclopropene ring was thought to be characteristic of the seed fat of *Sterculia foetida* (Sterculiaceae). However, a similar acid has since been located in the seed fats of the two species of Malvaceae. This contrasts with other members of the Malvaceae family which normally elaborate seed oils containing palmitic, oleic, and linoleic acids as the main constituents.

The correlation between the fatty acid composition of seed oils and the botanical classification thus deserves serious consideration but is not acceptable as an unequivocal guide as to the family, genus, or species to which a plant belongs.

V. Fatty Acid Composition and Mode of Combination

Within the same organism and even within the same tissue, wide differences in the fatty acid composition of different types of lipids are found. In animal tissues, for example, the phospholipids usually contain more of the higher molecular weight fatty acids than do the glycerides. Again the waxes in plants contain as a rule the higher saturated acids, while the glycerides and phospholipids are usually rich in oleic and linoleic acids. Differences in fatty acid composition are also observed between the different kinds of phospholipids.

In Section IV the fatty acids of the main depot fats, which are predominantly glyceridic, have been discussed. The fatty acids of waxes and phospholipids will be described in the present section.

A. Fatty Acids of Waxes and Related Substances

As mentioned in Section II,B, waxes are defined as the higher fatty acid esters of the higher fatty alcohols. The naturally occurring waxes, however, usually have in addition to esters many other components such as free fatty acids, free fatty alcohols, hydrocarbons, and sterols. Hydroxy acids are often present, giving rise to estolides and to lactones. The amounts of associated compounds vary with the source from which the wax has been derived. Although often present in only minor proportions, waxes are of widespread occurrence in plant life. They occur in tubercle bacilli in considerable amounts. In the higher plants they are usually found in the outer skin of leaves, stems, flowers, and fruits, as well as to some extent in the tissues. Waxes are not so common in animals, but occasionally they form considerable deposits, as in the head of the sperm whale, in the preen gland secretions of birds, and in the skin and hair lipids of animals.

The unique high molecular weight mycolic acids, which have been referred to in Section IV,D,1,a, serve to characterize the waxes of the acid-fast bacteria, as distinct from waxes found in other forms of life.

Table XIV shows the range in plant and insect waxes of *n*-saturated acids (C_{24}–C_{34}) the presence of which had been satisfactorily established (*274*). At that time (1934) the method for the resolution of the constituent higher fatty acids had not been well developed. It has since become obvious, mainly from the work of Murray and Schoenfeld (*23*) on carnauba wax that the fatty acids of waxes are more complex than was formerly realized and include all the *n*-even-numbered carbon saturated and ω-hydroxy acids from C_{18} to C_{30}. The ω-hydroxy acids were estimated to comprise 60% of the total. The results of Findlay and Brown (*275*) similarly show that several other hard vegetable waxes, including caranda, candelilla, and ouricury, also contain hydroxy acids, suggesting that the occurrence of ω-hydroxy acids in plant waxes is widespread (*23*).

TABLE XIV
THE FATTY ACID CONSTITUENTS OF PLANT AND INSECT WAXES (*274*)

Wax	*n*-Saturated fatty acids					
	C_{24}	C_{26}	C_{28}	C_{30}	C_{32}	C_{34}
Insect waxes						
Lac wax (*Coccus lacca*)				+	+	+
Cochineal wax (*Coccus cacti*)				+		
Chinese insect wax (*Coccus cerifera*)		+	+	+		
Beeswax (*Apis mellifera*)	+	+	+	+	+	+
Leaf cuticle waxes						
Carnauba wax		+	+	+	+	+
Candelilla wax			+	+	+	+
Raphia wax						
Pisang wax						
Fruit and seed coat cuticle waxes						
Apple cuticle wax		+	+	+	+	
Cotton wax	+	+	+	+	+	+
Leaf waxes (noncuticular)						
Brussels sprout		+	+			
Cabbage						
Swede turnip						
White mustard		+	+	+		
Cactus						
Tobacco						
Wild white clover		+	+	+		
Spinach	+	+				

The seed fat of *Simmondsia californica* (Buxaceae) is unique in that it consists of wax esters of higher unsaturated alcohols with higher unsaturated fatty acids, glycerides being entirely absent. The main fatty acid constituent is eicos-11-enoic acid (*263*).

In the animal kingdom, waxes are of sporadic and infrequent occurrence. Perhaps the best-known wax is the sperm oil obtained from the head and blubber of the sperm whale, *Physeter macrocephalus*, the fatty acids of which consist of C_{10}–C_{18} *n*-saturated and C_{14}–C_{22} unsaturated acids. The fatty acid composition (cf. Section IV,B,2) resembles that of a typical marine fish oil, but the average molecular weight of the fatty acids, which are largely monoethenoid, is lower (*167*).

The lipid secretions of the skin and hair, including wool, as well as those of the preen glands of birds, appear to consist largely of waxlike substances, though glycerides may also be present in some cases. The presence in the waxlike secretions of a wide range of straight-chain, odd- and even-numbered carbon fatty acids, saturated and unsaturated, as well as branched-chain acids has been shown. As will be discussed in Section VI, the presence of this unusual range of straight- and branched-chain acids, which varies in different species, serves to distinguish these waxlike substances from those found elsewhere.

The evidence considered in the present section points to some differentiation of fatty acid distribution in waxes in the different forms of life. In the acid-fast bacteria, the waxes are characterized by the presence of the unique mycolic acids, the mycolic acids varying perhaps in the different strains and types of acid-fast bacteria. In the plant and insect world, the waxes contain the higher *n*-saturated acids as well as, in some cases at least, the corresponding ω-hydroxy acids. However, in the seed wax of *Simmondsia californica*, the occurrence of an unsaturated fatty acid, eicos-11-enoic acid, forms a striking exception. In the higher animals the formation of a wax with the fish oil type of fatty acids in the main fatty depots of the sperm whale provides a parallel in the animal kingdom. The waxlike secretions of the hair, skin, and preen glands of the higher animals afford a fatty acid distribution that is not paralleled by the waxes occurring in other forms of life.

Related to the waxes are the cholesterol esters, which are of widespread occurrence, usually in minor amounts, in animal tissues. However, in blood plasma they are a major lipid constituent. The fatty acids present in cholesterol esters in blood plasma (*276*, *277*) and in liver (*278*) are characterized by their exceptionally high content (approximately 90%) of unsaturated acids consisting chiefly of linoleic acid. However, when di- and polyenoic acids are restricted in the diet, the amounts of these acids in the plasma and liver cholesterol esters are somewhat reduced (*279*). The fatty acid distribution in cholesterol esters is thus entirely different from that of the glycerides and phospholipids, which contain palmitic and oleic acids as the main constituents. In the flesh of the haddock cholesterol esters have also been found to be notably rich in highly unsaturated fatty acids as compared with those present in other lipids (*280*).

B. FATTY ACIDS OF PHOSPHOLIPIDS

In connection with these studies, most of the work on the types of fatty acids has been on phospholipid fractions separated as a rule from the corresponding glycerides by precipitation in acetone solution. Individual phospholipid species have also been investigated, but it is possible in some instances that the preparations are not representative of the particular phospholipid as it naturally occurs. The available methods of purification tend to remove or to select a fraction containing one type of fatty acid in preference to another.

1. *Phospholipids of Animals*

It will be seen from Table XV that the fatty acid composition of the phospholipids of various species follows a similar pattern and that, as compared with depot fats, differences in fatty acid composition between the phospholipids of aquatic species and those of higher land mammals are much less marked. The highly unsaturated C_{20} to C_{22} acids characteristic of fish depot fats are present only in slightly lower proportions in the phospholipids of higher land mammals as compared with fish. As earlier mentioned, however, the highly unsaturated acids of aquatic life are mainly of the linolenic type, whereas those of the higher land mammals are mainly of the linoleic type (*41*).

In fish the fatty acid composition of the corresponding phospholipids and glycerides is similar. In the higher land animals, however, the phospholipids, as compared with the corresponding glycerides (see Tables III and XV), have more of the higher molecular weight fatty acids, including especially the C_{20} and C_{22} highly unsaturated acids, but less palmitic and hexadecenoic acids. In particular the liver phospholipids, as compared with the depot fats, show rather pronounced differences as the latter have not more than traces of C_{20} and C_{22} highly unsaturated acids.

The main points of difference between the liver phospholipids, liver glycerides, and depot fats of the higher land mammals may be illustrated by consideration of the fatty acid composition of the horse lipids (*183*).

In addition to the differences shown in Table XVI very marked

TABLE XV
The Component Fatty Acids (Weight %) of Animal Phospholipids

Source	Saturated				Unsaturated				
	C_{14}	C_{16}	C_{18}	C_{20} and above	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Fish									
Ling (*Genypterus blacodes*), Roe (*281*)	1.3	25.0	0.9	—	—	2.1 (2.0 H)	20.2 (2.7 H)	34.4 (7.1 H)	16.1 (10.0 H)
Groper (*Polyprion oxygeneios*), liver (*166*)	←——18.5——→				—	16.9 (2.0 H)	19.6 (2.4 H)	31.1 (6.6 H)	13.9 (6.0 H)
Shark (*Galeorhinus australis*), head and body (*161*)	0.3	9.9	13.3	1.4	1.1 (2.0 H)	4.3 (2.0 H)	22.8 (2.3 H)	16.1 (2.5 H)	30.8 (10.0 H)
Amphibian									
Frog, liver (*282*)	←——25.0——→				←——42——→			←——33.0——→	
Reptile									
Greek tortoise, liver (*283*)	—	15.0	10.0	—	—	10.0 (2.0 H)	48.0 (2.8 H)	17.0 (6.0 H)	
Bird									
Hen, egg (*284*)	0.3	27.9	16.1	1.8	—	0.8 (2.0 H)	39.8 (2.4 H)	7.1 (6.8 H)	6.2 (8.4 H)
Mammals									
Nonruminants									
Pig, liver (*285*)	—	12.1	15.4	1.8	—	4.8 (2.0 H)	39.9 (2.2 H)	24.1 (6.5 H)	1.9 (6.5 H)
Horse, liver (*183*)	—	10.8	20.7	4.6	—	2.0 (2.0 H)	50.6 (3.4 H)	10.4 (5.7 H)	0.9 (5.7 H)
Ruminants									
Ox, liver (*286*)	—	12.5	27	—	—	5 (2.0 H)	27 (3.0 H)	18 (5.3—8.2 H)	10.5
Sheep, liver (*285*)	—	12.6	21.8	0.8	—	8.9 (2.0 H)	27.8 (3.1 H)	23.6 (6.9 H)	4.5 (10.5 H)

TABLE XVI

THE FATTY ACID COMPOSITION (WEIGHT %) OF THE DEPOT FAT, LIVER GLYCERIDES, AND LIVER PHOSPHOLIPIDS OF THE HORSE (*183*)

Source	Saturated				Unsaturated				
	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Depot fat	2.4	29.7	4.3	0.2	1.4 (2.0 H)	6.5 (2.0 H)	52.4 (3.4 H)	3.1 (3.3 H)	—
Liver glycerides	1.7	25.2	5.3	—	1.6 (2.0 H)	8.3 (2.0 H)	52.8 (4.5 H)	3.4 (6.2 H)	1.7 (6.0 H)
Liver phospholipids	—	10.8	20.7	4.6	—	2.0 (2.0 H)	50.6 (3.4 H)	10.4 (5.7 H)	0.9 (5.7 H)

TABLE XVII

THE COMPONENT FATTY ACIDS (WEIGHT %) OF PHOSPHOLIPIDS AND GLYCERIDES OF SEEDS AND LEAVES

Source	Saturated					Unsaturated						
	C_{14} and below	C_{16}	C_{18}	C_{20}	C_{22}	C_{14} and below	C_{16}	C_{18}			C_{20}	C_{22}
								Oleic	Linoleic	Linolenic		
Phospholipids												
Rapeseed (*288*)	1	8	—	—	2	—	2	22	42	—	—	23 (Erucic)
Peanut (*288*)	—	16.2	2.8	7.1[a]	—	—	—	47.1	22.7	—	4.1	
Cottonseed (*288*)	—	17.3	7.3	2.8	—	—	1.5	20.3	44.4	—	6.4	
Sunflower (*288*)	—	14.7	5.1	9.5	—	—	—	19.3	45.9	—	5.5	
Soybean (*288*)	—	11.7	4.0	1.4	—	—	8.6	9.8	55.0	4.0	5.5	
Soybean lecithin (*289*)	—	15.6	6.3	—	—	—	—	13.0	63.0	2.0	—	
Linseed (*288*)	—	11.3	10.6	—	—	—	3.5	33.6	20.4	17.4	3.2	
Ryegrass (*Lolium perenne*) (*290*)	1.3	12.6	0.6	18.1	—	—	1.8	5.6	20.2	37.8	2.0	
Clover leaf (*Trifolium repens*) (*290*)	0.3	12.6	0.8	9.2	—	—	7.6	10.1	28.6	30.8	—	
Rape leaf (*Brassica napus* L.)												
Glycerophospholipids (*290*)	—	13.8	6.1	5.9	—	—	4.9 (2.0 H)	←69.3→ (4.4 H)			—	—
Phosphatidic acid (*290*)	—	19.7	2.1	—	—	—	7.1 (2.0 H)	←71.1→ (4.0 H)			—	—

TABLE XVII—*Continued*

Source	Saturated					Unsaturated						
	C_{14} and below	C_{16}	C_{18}	C_{20}	C_{22}	C_{14} and below	C_{16}	C_{18}			C_{20}	C_{22}
								Oleic	Linoleic	Linolenic		
Glycerides												
Rapeseed (*288*)	—	2	—	—	1	—	—	17	29	—	—	51
Peanut (*288*)	—	8.3	3.1	—	6.6	—	—	56.0	26.0	—	—	—
Cottonseed (*288*)	1.4	23.4	1.1	1.3	—	—	2.1	22.9	47.8	—	—	—
Sunflower (*288*)	—	5.6	2.2	0.9	—	—	—	25.1	66.2	—	—	—
Soybean (*288*)	0.3	9.8	2.4	0.9	—	—	0.5	28.9	50.7	6.5	—	—
Linseed (*288*)	0.2	5.4	3.5	0.6	—	—	—	18.8	24.2	47.3	—	—
Ryegrass (*Lolium perenne*) (*89*)	1.8	10.6	1.5	0.4	—	0.7	4.1	4.6	11.6	62.8	1.9	—
Clover leaf (*Trifolium repens*) (*290*)	1.1	6.5	0.5	2.0	—	0.8	2.5	6.6	18.5	60.7	0.8	
						(2.0 H)	(2.0 H)		(5.4 H)		(3.0 H)	
Rape leaf (*Brassica napus* L.) (*290*)	←16.0→					0.7	11.3	←72.0→				
		(Mainly palmitic)				(2.0 H)	(6.0 H)		(5.4 H)			

[a] 4.6% of C_{20}, C_{22}, and C_{24} acids; 2.5% of C_{26} acids.

differences in the distribution of dienoic and trienoic acids between the phospholipid and glyceride fractions have been found. The ratios of C_{18} triene:diene in the depot glycerides, liver glycerides, and liver phospholipids of the horse were reported as 4.22, 2.39, and 0.13, respectively (*183*). Similar, but not so marked, differences are found also in the lipids of the rabbit (*287*).

2. *Phospholipids of Plants*

In general the range and types of fatty acids in the plant phospholipids (see Table XVII) are similar to those of the corresponding glycerides. The amounts of unsaturated C_{20}–C_{22} shown in Table XVII have been calculated from the saponification equivalents of the residual fractions obtained during ester fractionation. Klenk and Debuch (*291*) reexamined the phospholipids of soybean oil and rapeseed oil but were unable to substantiate these findings. The acids from the ether-insoluble polybromides from the phospholipid fatty acids, when hydrogenated, yield pure stearic acid. It is therefore unlikely that the highly unsaturated C_{20} and C_{22} acids characteristic of animal phospholipids are present in plant phospholipids.

In plants, the seed and leaf phospholipids are shown to contain the same fatty acids as are present in the corresponding glycerides, albeit in slightly different proportions. The phospholipids as compared with the glycerides tend to contain more saturated acids, particularly palmitic acid. There is more linoleic acid in phospholipids than in glycerides but, linolenic acid, when present, is concentrated in the glycerides.

3. *The Fatty Acid Composition of Specific Phospholipids and Related Substances*

Earlier work in this field involved the separation and purification of individual phospholipids by precipitation methods. The purified phospholipid thus obtained, often in low yield, was probably not representative in its fatty acid composition of the material as it existed in the tissues. However, the more recent work involving the use of chromatography and countercurrent extraction is perhaps less defective in this respect.

In connection with studies on the flesh lipids of fish, Lovern and Olley (*292, 293*) have shown that the phospholipids of haddock flesh, which contains very little glyceride, have a high content of highly unsaturated C_{20} and C_{22} acids, but no hexadecenoic acid. This acid is, of course, usually found in quantity in fish oils. The fatty acid composition of the main phospholipid component—lecithin—is compared in Table XVIII with that of the main depot fat.

TABLE XVIII
Comparison of the Fatty Acid Composition (Weight %) of Lecithin of Haddock Flesh with That of the Liver Oil

Source	Saturated				Unsaturated				
	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Liver oil (*157*)	4.3	14.1	0.3	—	0.5	12.4 (2 H)	30.5 (2.6 H)	29.3 (6.0 H)	8.6 (7.3 H)
Flesh lecithin (*293*)	—	18	7	2	—	—	14 (4.2 H)	35 (7.6 H)	24 (10.8 H)

TABLE XIX
The Component Fatty Acids (Weight %) of Individual Phospholipids

	Saturated				Unsaturated				
	C_{16}	C_{18}	C_{20}	C_{22}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
Ox heart lecithin (*294*)	34.2	7.3	—	—	8.4	42.6	7.5	—	—
Ox heart acetalphospholipid (*294*) (plasmalogen)	3.5	0.8	—	—	6.4	72.1	17.2	—	—
Brain phosphatidylethanolamine (*295*)	9.7	9.7	1.5	0.9	2.3	34.7	15.1	24.4	1.7
Brain phosphatidylserine (*295*)	3.2	32.7	—	—	—	51.6	7.1	5.4	—
Egg yolk phosphatidylcholine (*296*)	28	14	—	—	4	45	5	4	—
Egg yolk phosphatidylethanolamine (*296*)	20	40	—	—	1	20	9	10	—

The fatty acid compositions of the individual phospholipids isolated from mammalian tissues are collected in Table XIX. The results shown in Table XIX indicate very wide differences in fatty acid composition of individual phospholipids from the same tissues. It is perhaps to be expected that the acetalphospholipid or plasmalogen fraction with the fatty acids attached to the β-hydroxyl group of glycerol should be largely unsaturated (see Section II,B,3,d).

In the brain and egg lipids notable differences in fatty acid composition are shown between the phospholipids from the same tissues. In brain, phosphatidylethanolamine as compared with phosphatidylserine shows a much higher content of C_{20} and C_{22} unsaturated fatty acids. In studies on egg yolk phospholipids, the phosphatidylethanolamine and phosphatidylcholine were almost quantitatively separated. The phosphatidylethanolamine fractions as compared with the phosphatidylcholine are shown to be much richer in stearic acid. The lesser amounts of C_{18} unsaturated acids found in phosphatidylethanolamine as compared with phosphatidylcholine consist largely of linoleic acid. In phosphatidylcholine, C_{18} unsaturated acids are mainly oleic acid. The results in Table XIX show a diversity in fatty acid composition between individual phospholipids as well as between the same kind of phospholipid, e.g., phosphatidylethanolamine in brain as compared with that in egg yolk. The former is much richer in unsaturated fatty acids but contains less stearic acid.

Earlier investigations carried out on sphingomyelin show that the fatty acid present in this lipid is not solely lignoceric as is sometimes reported [cf. Hutt (*297*)]. For example, Rennkamp (*298*) records the following fatty acid composition (weight %): stearic, 46; nervonic (tetracos-15-enoic) and lignoceric, 34; palmitic, 2; arachidic, 2; behenic, 6, and hexacosenoic, 10.

In the cerebrosides there are four different types of galactolipids [cf. Deuel (*299*)], each of which contains a different fatty acid. Cerasine contains lignoceric, phrenosine, cerebronic (α-hydroxytetracosanoic) (*300*), nervone, nervonic (tetracos-15-enoic) (*301*), and oxynervone, oxynervonic (α-hydroxytetracos-15-enoic) acid (*302*).

4. *General Conclusions Concerning the Types and Distribution of Fatty Acids in Phospholipids*

The somewhat meager data concerning the fatty acid distribution of phospholipids in the various forms of life restrict the possibility of arriving at any firm generalizations. Nevertheless it is evident that the differences found between the fats of land plants and of fish are preserved in the phospholipids. The polyunsaturated C_{20} and C_{22} acids, which are major constituents of fish phospholipids, are not present in plant phospholipids. On the other hand, the highly unsaturated C_{20} and C_{22} acids are found in considerable amounts in the phospholipids of the higher land mammals, so that the differences in fatty acid composition between the higher land mammals and fish are not so apparent as in the glycerides.

The examination of individual phospholipids shows a striking variation in fatty acid composition with each kind of phospholipid.

Even more outstanding is the presence of distinctive high molecular weight fatty acids (not present in the glycerophospholipids) in the related sphingomyelin and cerebrosides. As sphingolipids have not been found in plant tissues, these compounds, together with their unusual fatty acids, afford a sharp distinction in lipid composition between the vegetable and animal kingdoms.

VI. Variations in Types and Distribution of Fatty Acids in Different Parts of the Organism

Variations in the nature of the lipids of different parts of the organism may be quite extensive. Not only are there variations in fatty acid composition and types of fatty acids, but also the kinds and proportions of the lipids themselves may change. The depot fats of mammals consist largely of triglycerides. In brain tissues the fatty acids are combined as phospholipids and cerebrosides, glycerides being almost entirely absent. The fatty acids are of relatively high molecular weight and include polyunsaturated C_{20} and C_{22} acids, in contrast to palmitic, stearic, and oleic acids, which form the main fatty acid constituents of the depot fats. In nonadipose tissues, such as liver, muscle, and blood plasma, phospholipids are often more abundant than triglycerides and, in addition, cholesterol esters are present (*303*).

The lipids from skin and hair of some mammals, including man, guinea pig, mouse, rabbit, and rat, appear to be largely nonglyceridic, and the fatty acids are either present in the free form or combined as sterol or wax esters (*304, 305*). In fish, the liver not infrequently forms the main site of fat storage, fatty acids being present as triglycerides. Under these circumstances, the body lipids may contain, as in the cod, a small amount of triglycerides, such lipids as are present (less than 1% of the fresh tissue) consisting largely of phospholipids (*306, 307*).

In plants, the fatty acid composition of the fruit-coat fat bears little relationship to that of the seed fat. The lipids of the fruit-coat fats and the seed fats are largely triglycerides, but in the leaf the lipids contain mainly galactolipids in place of triglycerides, the remainder consisting of unsaponifiable matter and phospholipids. Moreover, the composition of the fatty acids may differ considerably from that of the seed fat (*224*).

In making comparisons between the types and distribution of fatty acids in different parts of the same organism, it sometimes happens that the fatty acids concerned are present in different combinations. Nevertheless, it is considered profitable to make comparisons in fatty acid composition, bearing in mind the different types of combination.

In this section, owing to the rather limited information on the distribution of fatty acids in the different parts of plants by comparison with corresponding data on animals, it is convenient to reverse the procedure followed previously in this chapter and to deal with the plants first.

A. Variations in Fatty Acid Composition in Different Parts of Plants

The differences in fatty acid composition between the fruit-coat and seed fats of the same species have already been referred to. It is not intended to cover all the literature in this field, which is dealt with by Hilditch (*3*), but merely to illustrate with a few examples.

TABLE XX

Comparison between the Fatty Acid Composition (Weight %) of Fruit-Coat and Seed Fats of the Same Species

Species	Saturated				Unsaturated		
	C_{12}	C_{14}	C_{16}	C_{18}	Oleic	Linoleic	Linolenic
Fruit-coat fats							
Sapium sebiferum (*308*) (Euphorbiaceae)	1.5	3.4	72.1	1.6	20.4	1.0	—
Elaeis guineensis (*309*) (Palmae)	—	1.2	43.0	4.4	40.2	11.2	—
Seed fats							
Sapium sebiferum (*310*) (Euphorbiaceae)	—	—	9	3	8	25	51[a]
Elaeis guineensis (*272*) (Palmae)	45.2[b]	18.6	8.5	2.5	15.1	2.1	—

[a] Also 4% decadienoic acid.

[b] Also 2.4% octanoic acid, 3.7% decanoic acid, and 1.9% arachidic acid.

The examples given in Table XX show that the seed and fruit-coat fats from the same species not only differ markedly in their fatty acid composition, but also in the types of fatty acids present. The seed fat of *Sapium sebiferum* contains 51% linolenic acid, together with 4% of decadienoic acid, neither of which is found in the fruit-coat fat. Similarly, lauric acid, the major constituent of palm kernel oil, is absent from the fruit-coat fat.

Unfortunately there are few data permitting comparisons between the leaf fats and seed fats of the same species. However, as earlier mentioned, the leaf fats of the Gramineae contain linolenic acid as their main fatty acid whereas the seed fats of various species of this family seldom contain more than 10% of this acid but consist largely of oleic and linoleic acids (*3*).

The leaf fats of *Brassica napus* also appear to consist largely of linolenic acid with some hexadecatrienoic acid. In the seed fats of *Brassica* species generally, erucic acid is the major fatty acid constituent, but this acid was not found in the rape leaf lipids (*32*). This rather meager evidence points to the absence of correlation between the fatty acid constituents in different parts of the plants, and would be consistent with the elaboration of specific fatty acid types *in situ* without translocation to the different parts of the plant. The types and amounts of fatty acids in different parts of the same plant provide, therefore, a relatively unexplored field, which, on further study, could not fail to yield interesting data.

B. Variations in Fatty Acid Composition in Different Parts of Animals

In aquatic animals different species of fish vary in the distribution of their fat reserves. In some the fats are diffused more or less evenly throughout the organs and tissues whereas in others they are concentrated in the liver. Among fishes with a diffuse system of fat storage in which different fatty depots have been examined for fatty acid composition, are the salmon (*Salmo salar*) (*169, 311, 312*), sturgeon (*Acipenser sturio*) (*170*), and tunny (*Thunnus thynnus*) (*313*). These fishes, in the various depot fats, show no very marked differences in fatty acid composition. In some marine species, such as the New Zealand groper (*Polyprion oxygeneios*) (*166*) and the South African jacopever (*Sebastichthys capensis*) (*165*), in which the liver is also a secondary fat depot, the liver oil fatty acids are usually high in C_{16} and C_{18} unsaturated acids, but low in C_{20} and C_{22} unsaturated acids. The main fat depots, on the other hand, exhibit the normal marine type of fatty acid composition (see Table XXI).

In fishes in which fats are concentrated in the liver, such as the New Zealand ling (*Genypterus blacodes*) (*281*), it was found that the roe and visceral oils are somewhat richer in C_{20} and C_{22} unsaturated acids than the liver oils, but the differences are not marked. Similarly, in

TABLE XXI

The Component Fatty Acids (Weight %) of the Depot Fats of the Groper (*Polyprion oxygeneios*) *(166)* and of the Jacopever (*Sebastichthys capensis*) *(165)*

Source	Saturated				Unsaturated					
	C_{14}	C_{16}	C_{18}	C_{20}–C_{22}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
Groper										
Liver	1.9–2.4	19.3–23.0	3.3–3.4	—	0.1–1.6 (2.0 H)	17.3–23.2 (2.0 H)	39.3–45.2 (2.3–2.5 H)	7.0–9.1 (5.9–6.3 H)	Trace–4.0 (6.0–6.3 H)	—
Head	3.0	16.0	3.1	—	1.1 (2.0 H)	13.8 (2.0 H)	30.8 (2.6 H)	18.5 (6.2 H)	13.7 (9.2 H)	—
Jacopever										
Liver	1.2	11.6	3.9	0.4	0.6 (2.0 H)	13.5 (2.0 H)	46.3 (2.3 H)	12.7 (6.3 H)	7.5 (8.7 H)	2.4 (? H)
Head	2.6	16.3	2.1	0.8	0.9 (2.0 H)	11.8 (2.0 H)	30.3 (3.0 H)	18.8 (6.8 H)	15.5 (9.6 H)	1.0 (? H)
Body	2.6	13.8	1.8	0.2	2.3 (2.0 H)	12.4 (2.0 H)	28.5 (2.4 H)	21.6 (7.0 H)	16.8 (9.5 H)	—

the school shark (*Galeorhinus australis* MacLeay) (*161*), in which the liver is the main fat depot, the head and body oils are not appreciably different in fatty acid composition from the liver oils.

In the sperm whale, the main fat reserves are in the head and the blubber and are present largely as esters of higher fatty alcohols. The unsaturation is almost confined to the monoethenoid state, and the average molecular weight of the fatty acids is lower than in ordinary whale oil. The relative absence of C_{22} acids is noteworthy (*167*). The partial analysis of sperm whale liver oil by Tsujimoto and Kimura (*314*) suggests that this depot has a fatty acid composition of the normal marine type. In the porpoise and the dolphin the main depots, including the head, body, and jaw, contain considerable proportions of isovaleric acid, together with unusually high proportions of C_{14} and C_{16} acids. These unusual features are absent from the organ fats, the composition of which corresponds more closely to that of typical marine fat (*168*) (see Table XXII).

The depot fats of land animals also show variations in composition according to site of deposition. However, no appreciable variations in fatty acid composition are observed between the abdominal, neck, and gizzard fats of the hen (*179*).

To explain the relatively high content of fully saturated glycerides in ruminant depot fats especially, Hilditch (*3*) has suggested that such fats are assembled to begin with on an even-distribution pattern, as in other plant and animal fats. Subsequently the preformed oleoglycerides are hydrogenated *in situ* to give fully saturated glycerides. In accordance with this suggestion it has become widely accepted that the differences between the internal fats, such as kidney fat, and external fats, such as outer back fat, rest on their respective contents of oleic and stearic acids. The analytical data published have also hitherto supported this view. In passing from the kidney fat to the outer back fat it is found, as a rule, that the percentage of oleic acid rises, while that of stearic falls. The other major constituent, palmitic acid, remains fairly constant. This is illustrated in Table XXIII showing the changes in composition of the depot fats of pigs with site of deposition. In the liver fat there is a further change in composition with the appearance of C_{20} and C_{22} unsaturated acids, while in the bone fat the amount of palmitic acid falls below the more or less constant level of 25–30% characteristic of the main depot fats.

Recently Dahl (*317*), from a survey of the composition of the depot fats of farm animals, considered that the changing properties of animal fats as we proceed from the internal to the external depots arise differently in different animals. In pigs, the difference between the peri-

TABLE XXII

VARIATIONS IN FATTY ACID COMPOSITION (WEIGHT %) OF THE DEPOT FATS OF THE SPERM WHALE (*Physeter macrocephalus*) AND THE PORPOISE (*Phocaena communis*)

Source	Saturated						Unsaturated					
	C_5	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Sperm whale												
Head (*167*)	—	3.5	16	14	8	2	4 (2.0 H)	14 (2.0 H)	15 (2.0 H)	17 (2.0 H)	6.5 (2.0 H)	—
Blubber (*167*)	—	—	1	5	6.5	—	—	4	26.5 (2.0 H)	37 (2.0 H)	19 (2.5 H)	1 (4.0 H)
Liver (*314*)	—	←25%→ (chiefly palmitic acid)					←42%→ (monoethenoid)				←23%→	
Porpoise												
Body	13.6	Trace	3.5	12.1	4.7	—	—	4.7 (2.0 H)	27.2 (2.0 H)	16.7 (2.8 H)	10.5 (4.8 H)	7.0 (4.9 H)
Head	20.8	—	4.1	15.8	7.5	0.2	—	4.6 (2.0 H)	20.8 (2.0 H)	15.2 (2.6 H)	9.4 (4.5 H)	1.6 (4.7 H)
Jaw	25.3	Trace	4.6	28.3	4.1	—	—	3.2 (2.0 H)	20.3 (2.0 H)	9.3 (2.6 H)	4.9 (4.9 H)	—
Fetus	1.2	—	—	14.9	0.6	—	—	12.3 (2.0 H)	48.1 (2.0 H)	15.4 (4.0 H)	7.5 (7.4 H)	—
Liver	—	—	—	—	7.6	5.5	—	—	6.1 (2.0 H)	42.5 (2.8 H)	27.3 (5.4 H)	11.0 (6.5 H)
Lungs	—	—	—	4.6	9.0	1.2	—	0.1 (2.0 H)	16.5 (2.0 H)	27.0 (2.4 H)	31.0 (3.3 H)	10.6 (5.4 H)
Heart	—	—	—	8.1	8.2	4.4	—	4.4 (2.0 H)	16.8 (2.0 H)	50.4 (3.6 H)	7.6 (5.4 H)	—

TABLE XXIII

COMPONENT FATTY ACIDS (WEIGHT %) IN THE DEPOT AND BONE FATS OF PIGS

	Saturated					Unsaturated					
	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	Oleic	Linoleic	C_{20}	C_{22}
Perinephric (*315*)	—	0.9	29.3	17.4	—	0.3	1.8	40.3	8.1	←—1.9—→	
Inner back (*315*)	0.1	0.8	27.5	15.1	—	0.2	1.7	44.2	7.3	←—3.1—→	
Outer back (*315*)	0.1	0.9	26.5	12.8	—	0.2	1.9	46.8	7.9	←—2.9—→	
Liver fat (*285*)	—	0.1	22.5	9.5	—	—	8.9	←—47.0—→		10.6	1.4
Bone fat (Sow, 4 years old) (*316*)	0.3	1.8	19.2	6.1	0.6	0.5	2.5	51.2	12.2	1.3	4.3

TABLE XXIV
Variations in the Composition of Ox Depot Fats

Source	Fatty acids (wt. %)											
	Saturated					Unsaturated						
								C_{18}				
	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	Monoene	Diene	Triene	C_{20}	C_{22}
Perinephric (*319*)	0.1	3.3	27.0	31.6	0.7	0.4	1.7	33.5	0.8	—	←0.9→	
Intramuscular (*319*)	—	1.1	26.2	14.6	0.9	0.4	5.4	48.6	1.0	—	←1.8→	
Intermuscular (*319*)	—	3.4	26.0	17.9	0.4	0.8	3.9	44.8	1.0	—	←1.8→	
Subcutaneous (*319*)	—	3.2	27.4	13.4	0.9	1.5	4.3	45.8	1.3	—	←2.2→	
Liver glycerides (*319*)	—	3.0	18.3	14.5	1.3	1.9	3.4	26.5	4.5	3.3	12.3 (7.8 H)	11.0 (? H)
Yellow marrow (*320*)	0.1	2.6	32.3	15.5	—	0.7	3.0	43.2	2.6	—	—	—
Neatsfoot oil (*321*)	—	0.7	16.9	2.7	0.1	1.2	9.4	64.4	2.3	0.7	←1.6→	
Brisket fat (*318*)	—	3.2	31.3	5.4	—	1.9	16.9	38.9	2.4	—	—	—

nephric and outer back fats results, in accordance with Hilditch's views, from an exchange in the proportions of stearic and oleic acids.

In cattle, however, Dahl (*318*) considers that the differences between the perinephric and external tissue fats arise from changes in the proportions of palmitoleic and stearic acid. This view is supported by the analysis of an ox brisket fat, which shows about 17% palmitoleic acid as compared with less than 2% normally present in the perinephric fat. It is also supported to some extent by the work of Shorland and Hansen (*319*) (see Table XXIV), though here the external tissue fat, as compared with the kidney fat, contains more oleic as well as palmitoleic acid to compensate for the reduced proportions of stearic acid. In the New Zealand sample of external tissue fat the stearic acid content is still relatively high (13.4%) as compared with only 5.4% found by Dahl (*318*). It is possible in the colder climates that the external tissue fats are more unsaturated and contain less stearic acid. This accords with the findings for pigs of Henriques and Hansen (*322*) where they show that the iodine value is related to the temperature at the site of fat deposition. It is conceivable that if the stearic acid content of the New Zealand sample were lower the palmitoleic acid content would be more in line with that found by Dahl (*318*).

The yellow marrow fat of the bone appears to correspond in its fatty acid composition to that of other depot fats, whereas neatsfoot oil prepared from the hoofs of oxen is low both in stearic acid and palmitic acid. Here the liquid nature of the fat is due to the high content of both oleic and palmitoleic acids. In the liver glycerides the content of palmitic acid is also low but is compensated for not by palmitoleic, but by C_{20} and C_{22} highly unsaturated acids.

In the sheep the variations between the external tissue fats and the perinephric fats, as in the pig, are largely the result of an exchange between oleic and stearic acids (see Table XXV).

The liver glycerides contain considerable proportions of C_{20}–C_{22} unsaturated acids with diminished proportions of palmitic acid. The hoof oil of sheep resembles that of oxen, and the lowered content of saturated acids is compensated for by high proportions of both palmitoleic and oleic acids. The hoof oil is the only fat in sheep with notable proportions of palmitoleic acid.

Dahl considers that the changes in composition of horse fats from the internal to external parts of the body are slight, and this view is supported by the data shown in Table XXVI. However, in line with beef brisket fat (see Table XXIV), horse wither fat was found by Dahl (*318*) to contain somewhat higher (14.2%) proportions of palmitoleic acid.

The examples given for horse fats are taken from New Zealand

TABLE XXV
VARIATIONS IN COMPOSITION OF SHEEP FATS WITH SITE OF DEPOSITION

Source	Fatty acids (wt. %)										
	Saturated					Unsaturated					
	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}			C_{20}–C_{22}
								Monoene	Diene	Triene	
External tissue fat (*83*)	0.4	3.4	27.8	14.7	—	0.4	1.6	46.3	4.8	—	0.6
Perinephric (*83*)	—	2.9	24.0	24.9	—	0.7	2.4	39.2	5.2	—	0.7
Depot (*323*)	1.3	3.5	25.0	22.2	0.7	0.5	1.7	42.0	2.2	—	0.9
Muscle (*323*)	0.2	4.1	24.6	15.4	0.7	0.5	2.6	45.1	5.0	—	1.8
Liver (glycerides) (*291*)	—	0.2	21.9	12.9	—	—	4.9	←——44.7——→			11.6 3.8
Bone, sheep (6-month-old lamb, mixed bones) (*316*)	0.9	4.9	18.9	8.3	0.3	1.2	5.5	54.7	3.9	1.5	Trace
Neatsfoot oil (*290*)	0.3	2.6	16.2	4.5	0.5	1.0	10.7	58.0	3.7	1.3	1.2

TABLE XXVI

THE FATTY ACID COMPOSITION (WEIGHT %) OF THE DEPOT FATS OF HORSES

Source	Saturated					Unsaturated						
	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}			C_{20}	C_{22}
								Monoene	Diene	Triene		
Fatty tissues (*183*)	—	2.4	29.7	4.3	0.2	1.4	6.5	32.5	3.8	16.1	3.1 (3.3 H)	
Muscle glycerides (*183*)	0.4	2.4	29.9	3.0	0.2	1.5	8.7	34.9	3.4	12.5	3.1 (4.4 H)	
Liver glycerides (*183*)	—	1.7	25.2	5.3	—	1.6	8.3	17.6	10.4	24.8	3.4 (6.2 H)	1.7 (6.0 H)
Bulk sample of bone oil (*324*)	0.2	2.8	25.2	4.6	—	0.7	8.1	35.1	5.3	17.3	0.7 (4.0 H)	
Hoof oil (*324*)	—	1.0	19.5	2.4	0.6	0.7	20.5	←	53.1 (3.5H)	→	2.2 (4.0 H)	
Mesenteric fat (*325*)	0.4	4.5	25.9	4.7	0.2	—	6.8	33.7	5.2	16.3	2.3	

pasture-fed animals. The fatty tissue, muscle, and liver samples are from the same animals, whereas the hoof and bone oils were taken from animals in the same district and are probably comparable. The mesenteric fat, however, is from a pasture-fed horse in England.

The composition of the fats is much more alike in the horse than in the fats from the different parts of sheep and oxen, and there appears to be little, if any, change in the contents of either stearic or palmitoleic acids. The hoof oils, however, show, as in oxen, a decreased content of palmitic and stearic acid, which is largely compensated for by the rise in palmitoleic acid.

C. Fatty Acids of Milk Fats

Investigations on milk fats, especially of ruminants, have revealed that they are derived mainly by synthesis from acetate in the udder, though the dietary fatty acids contribute to some extent [cf. Shorland (*89*)]. The synthetic activity of the mammary tissues leads to the formation of volatile fatty acids which appear to a varying degree in the milk fats of animals.

The milk fats of ruminants, including those of the cow, buffalo, goat, camel, and sheep [cf. Hilditch (*3*)] are notable for their high contents of lower (C_4–C_{10}) saturated acids (see Table XXVII). The fatty acid composition of these fats is generally similar, but cow milk fats have a somewhat higher content of butyric acid. On the other hand, the goat and sheep milk fats are notably rich in decanoic acid (about 10 moles % as compared with 2–3 moles % found in cow milk fat). The presence of these high proportions of volatile fatty acids gives to the ruminant milk fats a characteristic feature that differentiates them from the corresponding depot fats in which these acids are not present in more than trace amounts. In the horse and human milk fats lower volatile fatty acids are also present, but in much lesser amounts, so that they are more like depot fats than are the ruminant milk fats. Sow milk fat resembles fairly closely pig depot fats, and the milk fats of seals and whales, in which the lower volatile acids are absent, are virtually indistinguishable from a typical marine mammalian depot fat.

The results given in Table XXVII are based on ester-fractionation techniques which do not readily reveal the traces of *n*-odd numbered and branched-chain fatty acids now known to occur in ruminant milk fats (*332a*). These constituents have also been revealed in human milk fats by use of gas-liquid chromatography (*332b*).

D. The Fatty Acids of Sebum and Hair

The best-known of these secretions is wool wax, the fatty acid composition of which has been elucidated only within the past fifteen

TABLE XXVII

The Fatty Acid Composition (Weight %) of Milk and Depot Fats of Different Species

Source	Saturated									Unsaturated								
	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{10}	C_{12}	C_{14}	C_{16}	C_{18} Monoene	C_{18} Diene	C_{18} Triene	C_{20}	C_{22}
Cow																		
Milk (*326*)	3.5	1.9	0.7	2.1	1.9	7.9	25.8	12.7	1.5	0.1	0.2	0.6	2.4	34.0	3.7	—	1.0	—
Perinephric (*327*)	—	—	—	—	—	3.0	29.2	21.0	0.4	—	—	0.6	2.7	41.1	1.8	—	0.2	
Sow																		
Milk (*328*)	—	—	—	1.3	—	1.5	26.9	6.5	—	—	—	—	8.3	36.7	14.6	—	4.2	
Inner back (*82*)	—	—	—	—	—	3.1	29.2	11.9	—	—	—	—	5.9	49.3	0.5	—	0.1	
Horse																		
Milk (*329*)	0.4	0.9	2.6	5.5	5.6	7.0	16.1	2.9	0.3	0.9	1.0	1.8	7.5	18.7	7.6	16.1	5.1	
Mesenteric (*325*)	—	—	—	—	0.4	4.5	25.9	4.7	0.2	—	—	—	6.8	33.7	5.2	16.3	2.3	
Human																		
Milk (*330*)	0.4	0.1	0.3	2.2	5.5	8.5	23.2	6.9	1.1	0.1	0.1	0.6	3.0	36.5	7.8	0.4	3.3	
Depot fats (*187*)	—	—	—	—	0.1	2.7	24.0	8.4	—	—	—	0.2	5.0	46.9	10.2	—	2.5	
Seal, Gray Atlantic (*Halichoerus grypus*)																		
Milk (*331*)	—	—	—	—	—	2.8	16.4	2.8	—	—	—	1.7	12.7	36.6 (2.3 H)	—	—	13.6 (6.6 H)	13.4 (11.2 H)
Blubber (*332*)	—	—	—	—	—	3.4	8.9	1.8	0.2	—	—	2.0	15.6	33.4 (2.5 H)	—	—	18.7 (6.2 H)	16.0 (10.1 H)

TABLE XXVIII

Fatty Acid Composition (Moles %) of Sebum of Humans (*305*) and of Some Common Rodents (*304*)

Acid	*n*-Saturated					Monounsaturated (Diunsaturated, DU; highly unsaturated, HU)					Branched (Highly branched, HB)				
	Human	Guinea pig	Mouse	Rabbit	Rat	Human	Guinea pig	Mouse	Rabbit	Rat	Human	Guinea pig	Mouse	Rabbit	Rat
C_{10}	0.08	Trace	Trace	0.12	Trace	—	—	—	—	—	—	—	—	—	—
C_{11}	Trace	Trace	Trace	0.3	Trace	—	—	—	—	—	Trace	Trace	Trace	Trace	Trace
C_{12}	3.64	Trace	Trace	2.2	Trace	—	—	—	—	—	Trace	Trace	Trace	Trace	Trace
C_{13}	0.25	Trace	Trace	6.6	Trace	—	—	—	—	—	0.25	Trace	Trace	0.6	Trace
C_{14}	6.35	0.3	0.7	10.7	1.6	0.76	Trace	0.4	—	0.5	0.32	Trace	0.2	0.7	2.6
C_{15}	2.27	1.6	1.1	6.0	0.8	0.64 0.28(DU)	—	0.7	—	—	0.67 0.54(HB)	0.7	0.3	1.3	1.1
C_{16}	24.2	15.8	5.0	24.0	17.4	9.05	1.1	7.3	—	2.1	0.29	0.5	1.7	0.5 1.8(HB)	4.7
C_{17}	1.07	5.8	1.7	6.8	3.1	1.62 0.69(DU)	0.8	0.9	—	—	0.73 1.13(HB)	2.4	0.9	1.7 11.0(HB)	5.0
C[illegible]	8.0	4.0	3.9	8.2	8.9	35.6	7.7	10.0	3.7	11.3 3.1(DU) 1.7(HU)	1.8	Trace 3.1(HB)	0.7	— 14.3(HB)	11.3
C_{19}	—	2.2	2.2	—	—	—	3.1	—	—	—	—	9.8	1.0	—	3.1
C_{20}	—	—	9.2	—	—	—	—	—	—	—	—	4.3	5.1	—	6.8 4.1(HB)
C_{21}	—	—	6.1	—	—	—	—	—	—	—	—	—	2.5	—	—

years. Human hair fatty acid composition has likewise only recently been satisfactorily described. The advent of gas-liquid chromatographic techniques has permitted the determination of the fatty acid composition of the sebum from a number of other species where the collection of sufficient material for the application of the hitherto available techniques for the determination of fatty acid composition had proved to be an obstacle.

In addition to the figures given in Table XXVIII for the sebum of humans, guinea pigs, mouse, rabbits, and rats, very complete data are also available for wool grease, which appears to differ in its fatty acid composition from that of the sebum of the animals (see Table XXIX) by its absence of unsaturated acids. The wider range of higher molecular weight fatty acids shown for wool grease may be due to the analytical techniques used as the gas-liquid chromatographic techniques on which the results shown in Table XXVIII are based were not extended beyond C_{21}.

TABLE XXIX

FATTY ACID COMPOSITION (WEIGHT %) OF WOOL GREASE (*49*)

Acids	*n*-Saturated	Iso	(+)-Anteiso	α-Hydroxy
C_{10}	Trace	Trace	—	—
C_{11}	—	—	Trace	—
C_{12}	Trace	Trace	—	—
C_{13}	—	—	1	—
C_{14}	2.8	2.8	—	Trace
C_{15}	—	—	4.8	—
C_{16}	2.8	5.8	—	4.0
C_{17}	—	—	3.6	—
C_{18}	Trace	4.0	—	—
C_{19}	—	—	4.8	—
C_{20}	Trace	5.0	—	—
C_{21}	—	—	5.6	—
C_{22}	Trace	4.0	—	—
C_{23}	—	—	3.6	—
C_{24}	1	2.8	—	—
C_{25}	—	—	7.0	—
C_{26}	1	3.6	—	—
C_{27}	—	—	5.2	—
C_{28}	—	0.8	—	—
C_{29}	—	—	Trace	—
C_{30}	—	—	—	—
C_{31}	—	—	Trace	—

Wool wax consists for the most part of ester waxes in which the alcohols are a mixture of sterols (cholesterol, isocholesterol, and lanosterol). Glycerides are probably absent. In human hair fat, Nicolaides and Foster (*333*) have shown that in addition to free fatty acids there are present mono-, di-, and triglycerides and waxes. In addition, the acids combined as waxes possess a somewhat higher iodine value than those combined as glyceride or present in the free form.

The inverse relationship between the free fatty acids and total glyceride contents suggests that the free fatty acids, mono- and triglycerides, represent more or less complete stages of hydrolysis of triglycerides. On the other hand the fatty acids of the sebum of the rodents (*304*) shown in Table XXVIII were mainly present in the combined form as wax or sterol esters, and not more than traces of glycerol were formed on saponification. The analysis of the sebum of the rodents refers to the total fatty acids, but in the human sebum the values recorded in Table XXVIII are for the free acids only (*305*).

These values are not unlike those obtained earlier by Weitkamp *et al.* (*334*) for the free fatty acids of human hair fat. However, James and Wheatley (*305*) used gas-liquid chromatography, which permitted a sharper resolution than ester fractionation, thereby revealing the presence of small amounts of branched-chain fatty acids in addition to the odd- and even-numbered *n*-saturated and unsaturated acids reported by Weitkamp *et al.* (*334*).

The presence of unsaturated acids with double bonds mainly in the 6,7-position, and to a lesser extent in the 8,9-position, shows that human sebum is different from the depot fat, where the 9,10-position (of oleic acid) is the most common one (*334*). In the C_{18} unsaturated acids of human sebum, however, oleic acid is still the most abundant unsaturated acid.

The unsaturated acids of the sebum of rodents have not been examined in detail. Taking the results as a whole it will be seen from Table XXVIII that not only are the sebum fatty acids markedly different in composition between different species, but they are also markedly different in composition from their depot fats.

Considering the *n*-saturated acids, these are represented to the extent of about 8% in wool grease and consist chiefly of C_{14} and C_{16} acids with lesser amounts of C_{24} and C_{26} acids. On the other hand the *n*-saturated acids of human sebum form about 50% of the total and cover a range from C_{10} to C_{18} with C_{16} predominating. The saturated acids of the sebum of the guinea pig, mouse, and rat are present in lesser amounts than in the human sebum. The mouse sebum stands apart in containing predominantly the higher molecular weight *n*-saturated acids from C_{14} to C_{21}.

n-Unsaturated acids are apparently absent from wool grease, while in the rabbit sebum only 3.7% is present. The human hair fatty acids consist of nearly half their total as *n*-unsaturated acids with octadecenoic as the main constituent, together with hexadecenoic acid. In the guinea pig with its lower content of unsaturated acids octadecenoic followed by nonadecenoic, not present in the other sebum lipids, are the two prominent constituents. In the rat, octadecenoic is also the main *n*-unsaturated constituent, followed by *n*-hexadecenoic acid, the *n*-odd-numbered unsaturated acids being absent.

In wool grease the branched-chain acids make up the greater part of the total and cover a wide range of carbon numbers, more than 2% of each acid being found over the range C_{14} to C_{27}. In the sebum fats, other than wool grease, branched-chain acids are present in much smaller amounts. The rat sebum, however, is notable for its high content of C_{18} branched-chain acids and for the occurrence of polybranched C_{20} acids. The rabbit sebum has important quantities of polybranched C_{17} and C_{18} acids, and the most important branched-chain acid in the guinea pig sebum is C_{19}. The human sebum is comparable to that of the mouse with its low content of branched-chain acids, but the two kinds of sebum are differentiated by the differences in composition of the branched-chain constituents.

Other fatty secretions of considerable interest are those of the preen glands of birds. The coccygeal or preen glands are found in birds as a special organ in place of the sebaceous glands distributed over the whole body surface in mammals. Weitzel and Lennert (*335*) found that the ether-soluble substances of the preen glands of ducks consist essentially of equal parts of octadecanol and fatty acids (see Table XXX).

TABLE XXX

THE FATTY ACID COMPOSITION (WEIGHT %) OF THE PREEN GLAND LIPIDS (*335*)

Saturated								Unsaturated	
Normal		Methyl branched							
C_{16}	C_{18}	C_7	C_8	C_9	$(C_9–C_{12})$	$(C_{14}–C_{16})$	C_{18}	$(C_{14}–C_{18})$	C_{18}
17.3	1.3	30.2	4.3	1.4	9.0	11.8	12.2	1.1	11.4

The main branched-chain fatty acid was shown to be levorotatory 4-methylhexanoic acid (*336*, *337*). Similarly, in the preen glands of geese the fatty substances consisted largely of octadecyl esters of a levorotatory branched-chain C_{14} acid with more than one side methyl group, with smaller amounts of C_{13} acids. Palmitic, stearic, and oleic acids were also present (*338*).

E. Fatty Acids of Nonadipose Tissue Lipids

Included in this category are the liver lipids of mammals which, unlike those of fish, are not important fat depots. Their fatty acid composition has been discussed in Section V,B,1, where it was shown that the liver glycerides, and especially the liver phospholipids, are richer in highly unsaturated C_{20} and C_{22} acids than are the depot fats. The organ lipids, in general, appear to contain more highly unsaturated C_{20} and C_{22} acids than the depot fats, but there are few detailed fatty acid analyses upon which to base general conclusions. However, in Table XXXI is shown the fatty acid composition of the lipids of ox heart muscle and of ox adrenal phospholipids. Although beef adrenal phospholipids have been commonly used as a source for the preparation of arachidonic acid, Holman and Greenberg (*341*) have shown that many other mammalian tissues provide lipids rich in polyenoic acids. Gonadal tissue is rich in both hexaenoic and tetraenoic acids. Lamb testes lipids, for example, contain 15.6% hexaenoic acid and 10.3% tetraenoic acid. As sources of arachidonic acid the lipids from hog testes, ovaries, uterus, liver, and brain are superior to adrenal lipids.

TABLE XXXI
Component Fatty Acids (Weight %) of the Lipids of Heart Muscle and Adrenals of the Ox

Source	Saturated				Unsaturated			
	C_{14}	C_{16}	C_{18}	C_{20}	C_{16}	C_{18}	C_{20}	C_{22}
Heart muscle (*339*)								
Glycerides	—	22	20	—	12	45	—	—
Phospholipids	—	14	21	—	—	45	14	1
Adrenals								
Phospholipids (*340*)	1.2	23.8	11.1	2.0	—	40.2	22.2	—

Klenk and Dreike (*342*), using alkali isomerization and ozonolysis (*343*), have reported the occurrence of polyenoic acids in the glycerophospholipids of ox liver (see Table XXXII).

TABLE XXXII
The Polyenoic Acids of the Glycerophospholipids of Ox Liver (*342*)

C_{18}:	$\Delta^{9,12}$ and $\Delta^{9,12,15}$
C_{20}:	$\Delta^{5,8,11}$, $\Delta^{8,11,14}$, $\Delta^{5,8,11,14}$, and $\Delta^{8,11,14,17}$
C_{22}:	$\Delta^{5,8,11,14,17}$, $\Delta^{7,10,13,16,19}$, and $\Delta^{4,7,10,13,16,19}$

Using improved techniques involving the resolution of the fractions by countercurrent extraction, Klenk and Lindlar (*344*, *345*) have established with considerable certainty the constitution of the C_{20} and C_{22} polyenoic acids of human brain as the same as those shown in Table XXXII for the glycerophospholipids of ox liver, except that eicos-8,11,14,17-tetraenoic acid was not found, and the presence of docosapentaenoic acid was uncertain.

F. Blood Lipids

Information on blood lipids is rather meager. However, there are data concerning the composition of lipids of ox blood.

The distribution of fatty acids shows wide differences in the different fractions. In both series the triglycerides resemble in their composition the depot fats (cf. Table XXXIII) although the first sample contains an unusually high content of linoleic acid. The sterol esters which form the greater part of the total lipids are entirely different in composition. As mentioned in Section IV, they are notably rich in linoleic and total unsaturated acids. Thus the distribution of fatty acids in ox blood at least will differ from that of the main depot fats and tissues in the relatively higher content of linoleic and unsaturated acids generally.

G. General Conclusions

In plants it is clear even from the limited data available that in the different parts of plants there are different types of lipids. In the leaf, waxes and phospholipids are important constituents, in addition to galactolipids, whereas in the seeds the fatty acids are present very largely as triglycerides. The seed fats are not necessarily related to the leaf fats in their fatty acid composition. The predominant fatty acid of rapeseed—erucic acid—does not occur in the leaf, and conversely hexadeca-7,10,13-trienoic acid, a major constituent in the leaf galactolipids is apparently absent from the seed fat. Similarly, comparisons between fruit-coat and seed fats show that each part elaborates its own types of fatty acids.

In animals fed a diet low in fat, the depot fats invariably consist of 25–30% palmitic acid, together with stearic acid, usually in lesser amounts, and oleic acid. On the other hand the brain lipids as well as those of the nonadipose tissues usually contain considerable proportions of C_{20} and C_{22} highly unsaturated acids. These apparently arise from the dietary linoleic and linolenic acids. However, as mentioned in Section V, the sphingolipids contain longer-chain acids of the C_{24} and C_{26} series, including saturated, α-hydroxy substituted, and unsaturated acids, which are presumably synthesized in the tissues and are characteristic of animal life. The fatty secretions of skin, hair, and preen

TABLE XXXIII

The Fatty Acid Composition (Moles %) of Ox Blood Lipids

Lipid	% Total lipid	Saturated				Unsaturated					
		C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	Oleic	Linoleic	Linolenic	C_{20}
Kelsey and Longenecker (*276*)											
Free fatty acids	19.9	0.8	34.4	5.2	2.7	—	—	40.5	16.4	—	—
Glycerides	16.8	0.2	33.7	22.2	0.5	—	2.6	21.3	18.4	—	1.1
Sterol esters	46.0	—	11.1	3.3	0.3	—	4.2	7.9	61.7	9.2	2.2
Lough and Garton (*277*)											
Triglycerides	7.5	4.6	29.9	30.6	—	1.8	7.2	21.5	1.5	—	2.9
Sterol esters	81.8	4.4	1.6	2.1	0.8	—	11.2	6.1	35.8	32.0	6.0

glands similarly appear to differ markedly in their fatty acid composition from depot fats. The branched-chain fatty acids are prominent constituents and differ in type from those found in the depot fats. There is not enough evidence to show that each species elaborates in the secretions mentioned its own type of fatty acids, but evidently there are marked differences between some species. Thus, although a study of the fatty acid composition of the main depot fats of higher animals may serve merely to reveal the nature of the dietary fats, it is not impossible that studies on the fatty acid composition of the minor fatty depots and secretions will reveal interesting information permitting correlations to be made between chemical composition and the classification of species on morphological grounds.

VII. Theory of Evolution and Fatty Acid Composition

A. General Considerations

In the preceding sections it has been shown that the component acids of fats and other lipids tend to align themselves in groups according to their biological origin. It is now desirable to collate the evidence from these groups and determine what relationships, if any, exist between fatty acid occurrence and the place of organisms in the evolutionary scale of development. This will involve some interesting comparisons between the processes of evolution of fats and other lipids in plants and animals.

Hilditch (*346*) suggested in 1935 that "perhaps, when in the course of time sufficiently wide and detailed data have been collected, systematic description of natural fats will commence with those of the minute aquatic flora and fauna, will proceed to those of the larger aquatic denizens, and then to the two respective branches of land flora and land fauna." This prediction has, to some extent, been fulfilled, and it may now be said that the systematic treatment of natural fats according to their biological origin has been widely adopted. Furthermore, it is clear that many close parallelisms exist between biological classifications and the types of fatty acids present in the lipids of different species as originally described by Hilditch and Lovern (*140*). At the same time it is equally apparent that the fats from organisms such as yeasts or molds may resemble closely those of animals or seeds, thereby indicating the absence of correlation between biological classification and chemical composition. The occurrence of oleic, palmitic, and stearic acids in the lipids of most forms of life is perhaps explained by the operation of a similar mechanism of synthesis of fatty acids in plants and animals. This mechanism involves the successive condensation of acetyl CoA units as outlined in the Lynen fatty acid cycle (*80*). However, there appears to be some variation in the operation of the acetate condensation process. In plants (*128*) and some microorganisms (*138*) linoleic and linolenic acids, which do not occur in the endogenous fats of animals, are also formed by the successive condensation of acetate units, indicating the presence of mechanisms in plants (*123, 128, 129*) that are absent in animals. There is no substantial evidence that these more highly unsaturated acids are formed by the desaturation of saturated acids or of oleic acid. On the other hand, there is in animals a mechanism for the synthesis of the highly unsaturated C_{20} and C_{22} unsaturated acids from the dietary linoleic and linolenic acids by the addition of acetate followed by desaturation (*84, 85*). Such a mechanism is absent from land plants which do not elaborate the highly unsaturated C_{20} and C_{22} acids. On the other hand, the presence of such acids in aquatic plants suggests that such a mechanism must also operate here.

The many different types of acids in plant life, some of which are specific, such as the branched-chain acids of tubercle bacilli, petroselinic acid of the Umbelliferae, chaulmoogric acid of the Flacourtiaceae, to name a few, must surely be the result of the presence of diverse enzyme systems, the nature of which remains obscure.

It is not possible to make completely satisfactory comparisons between the total fatty acids in animals and plants, as only the depot fats, such as perinephric fat or seed fat, have been examined. The organ lipids such as those of the liver differ in composition from their depot fats, particularly in land mammals, and leaf fats differ from seed fats. Nevertheless, allowing for these defects it is useful to compare the distribution and nature of the fatty acids on the basis of the depot fats in different organisms.

B. The Fatty Acids of Depot Fats of Organisms

In comparing the fatty acids in the depot fats of different organisms it is convenient to consider the types of fatty acids separately. In the discussion below the amounts of fatty acids are expressed as weight percentages of the total fatty acids.

1. *n-Saturated Acids*

n-Saturated acids, usually mainly palmitic acid, are found in the lipids of nearly all living organisms. The lowest recorded value is 0.5% found in the fatty acids of the nettle (*Urtica dioica*) (*231*). The saturated acids in the seed fats range from 2% in the seed fats of the Canadian nasturtiums (*347*) to over 90% in some of the Palmae (*3*).

Examples of marked deviations in the composition of the saturated acids include those of the stearic-rich seed fats (Section IV,D,4,h), with up to 60% stearic acid as in *Garcinia indica* (*270*), the seed oils rich in myristic acid as in *Myristica fragrans* with 76.6% myristic acid (Section IV,D,4,i), the seed oils rich in lauric acid, as in *Cocos nucifera* (*272*) with 45.2% lauric acid and the *Ulmaceae* with 50–73% decanoic acid (Section IV,D,4,j). The amounts of higher saturated acids (Section IV,D,4,g) in seed fats seldom exceeds 10%, but in *Adhatoda vasica* (*269*) these acids become major constituents with 22.2% behenic and 10.7% lignoceric.

In the cryptogams, the *n*-saturated acids usually consist mainly of palmitic acid with lesser amounts of stearic acid. In yeasts and molds the amounts are often like those of mammals.

In the fats of aquatic plants and animals there is, as a rule, about 10–15% of palmitic acid with lesser amounts of myristic and stearic acids, but in the liver oils of some sharks the amounts of palmitic and stearic acid may rise, respectively, to 25 and 15%. In amphibians and reptiles (see Table III) a somewhat similar situation prevails. The composition of their saturated acids approaches that of nonruminant mammals in which palmitic acid comprises usually 25–30% and stearic acid 3–10%. In a few nonruminants, however, the amounts of stearic acid may rise to 20%, and in ruminants it commonly reaches this amount and may even exceed that of palmitic acid.

The distribution and amounts of *n*-saturated acids in the depot fats provides a limited basis for the classification of species.

The Palmae, Myristicaceae, and Ulmaceae are characterized by the high contents in their seed fats of lauric, myristic, and decanoic acids, respectively. Land mammals are characterized by the presence of 25–30% palmitic acid in their depot fats, which serves, as a rule, to distinguish them from the seed fats as well as from fats of aquatic origin, in which the amounts of palmitic acid are typically less than 20%. However, yeast and mold fats, which sometimes contain larger amounts of palmitic acid, cannot thus be distinguished from mammalian fats.

2. *n-Unsaturated Acids*

The importance of these constituents in lipids may be seen from the fact that in seeds oleic and linoleic acids are estimated to form 80% of the total fatty acids, while oleic acid probably accounts for not less than 40% of all the fatty acids synthesized (*348*).

The endogenous unsaturated acids of land mammals are almost exclusively oleic, with lesser amounts of palmitoleic acid.

In aquatic plants and animals, polyethenoid acids of the C_{18}, C_{20}, and C_{22} series are important constituents. They are usually based on the pattern of linolenic acid with the first double bond in position 3 with respect to the CH_3 group. There are up to five double bonds in the C_{20} series and up to six double bonds in the C_{22} series, each double bond being separated from the next by a single methylenic group. Somewhat similar C_{20} and C_{22} acids, based mainly on the linoleic pattern, are found in the nonadipose tissue glycerides and phospholipids of land mammals (*41*). In addition, in ruminants the microorganisms in the rumen hydrogenate the dietary linoleic and linolenic acids, giving rise to mono- and to a lesser extent diethenoid acids which are *trans* and positional isomers of oleic and linoleic acids (*121*).

Although as indicated above, oleic and linoleic acids are the main fatty acids in plant fats, nevertheless the occurrence, in addition, of a great diversity of unsaturated acids, makes it desirable to examine their nature more closely.

In the monoethenoid series Hilditch (*348*) has shown that many of the naturally occurring acids have a structure identical with one or other half of oleic acid, i.e., $CH_3\ (CH_2)_7\ CH=$ or $=CH\cdot(CH_2)_7\cdot COOH$. A well-known example is that of erucic (docos-13-enoic) acid, which is present in quantity in the seed fats of the Cruciferae and Tropaeolaceae. In three species of the genus *Ximenia* of the Olacaceae family the monoethenoid C_{20}, C_{22}, C_{24}, C_{26}, C_{28}, and C_{30} acids belonging to the series $CH_3\ (CH_2)_7\ CH{=}CH\ (CH_2)_n\ COOH$ have been isolated and found to comprise in all 25–33% of the total fatty acids (*266*). These features, together with the occurrence of ximenynic (octadec-11-en-9-ynoic) acid, provide a unique fatty acid composition.

In the seed fats of the Umbelliferae, petroselinic (octadec-6-enoic) acids shows a deviation in structure from the oleic type. This serves to characterize the Umbelliferous species in which petroselinic acid invariably occurs.

A further deviation is that of the occurrence of *cis*-vaccenic (octadec-11-enoic) acid as the main unsaturated acid of certain bacterial lipids including those of *Lactobacillus*, *Streptococcus hemolyticus*, and *Agrobacterium tumefaciens* (*206, 207*).

The nonconjugated dienoic acids are represented by linoleic (octadeca-9,12-dienoic) acid. Apart from the minor amounts of the dienoic acids in ruminant fats, the only other nonconjugated dienoic acid found in substantial amounts is hexacosa-17,20-dienoic acid, which occurs in the lipids of the sponge *Spheciospongia vesparia* (*155*). In addition may be mentioned the conjugated dienoic acid, deca-2,4-dienoic acid (*29*) and dodeca-2,4-dienoic acid (*247*), found in two species of Euphorbiaceae.

In the trienoic series linolenic (octadeca-9,12,15-trienoic) acid is found in many seed fats as well as in the lipids of some cryptogams. The related hexadeca-7,10,13-trienoic acid has been found in rape leaf, but not in the seed fats (*32*, *227*). Other nonconjugated trienoic acids include octadeca-6,9,12-trienoic acid found in the seed fats of *Oenothera biennis* (*234*, *235*).

In the seed fats of the Rosaceae, Euphorbiaceae, and Cucurbitaceae some of the species contain as major constituents α-eleostearic (octadeca-*cis*-9,*trans*-11,*trans*-13-trienoic) acid or similar conjugated acids including 18-hydroxy-α-eleostearic and parinaric (octadeca-9,11,13,15-tetraenoic) acid, replacing entirely or partly linolenic acid (*37*). As a further variation there occurs in *Calendula officinalis* (Compositae) octadeca-*trans*-8,*trans*-10,*trans*-12-trienoic acid (*250*). The occurrence of trienoic acids with conjugated double bonds in the seed fats of species of the families mentioned above provides a unique differentiation from the rest of the animal and plant world. However, the absence of such acids in some of the species somewhat reduces their value as a complete guide to their botanical classification.

The sporadic and rare occurrence of acids containing acetylenic bonds, such as tariric (octadec-6-ynoic) acid in the genus *Picramnia* (Simarubaceae) (*257*), 8-hydroxyoctadec-17-en-9,11-diynoic acid in *Onguekoa gore* (Olacaceae) seed oil (*258*) and octadec-11-en-9-ynoic acid (*43*, *44*) in *Ximenia* (Olacaceae) as well as in Santalaceae species (*260*, *261*) affords an interesting diversion, the value of which in botanical classification is not clear.

Several unusual hydroxy-substituted unsaturated acids of limited occurrence have been mentioned above. To these may be added ricinoleic (12-hydroxyoctadec-9-enoic) acid present as the main constituent of castor (*Ricinus communis*) (Euphorbiaceae) seed oil (*3*), and its isomer 9-hydroxyoctadec-12-enoic acid present in the seed oils of *Strophanus sarmentosus* (*25*) (Apocynaceae). The related oxygenated acid vernolic (12,13-epoxyoctadec-9-enoic) acid occurs in *Vernonia anthelmintica* (*26*) (Compositae), *Cephalocroton cordofanus* (Euphorbiaceae) (*245*), and in *Hibiscus esculentus* Linn. (Malvaceae) (*253*). The appearance of unusual hydroxy or epoxy acids in apparently isolated species in several different families would appear to show an absence of correlation between the occurrence of chemical constituents and biological classification.

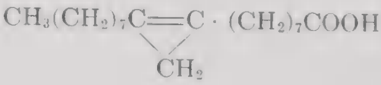
3. *Branched-Chain Acids*

The occurrence of branched-chain fatty acids in more than minor amounts is restricted. Isovaleric acid is found in quantity in the depot fats of the porpoise and dolphin but is absent from the liver and other organs (*168*). In ruminant depot fats there occur as minor constituents (+)-*anteiso* acids of the odd series and iso acids of the odd and even series within the range of C_{13} to C_{18} (*46–48*). The (+)-anteiso acids also occur in wool grease in the odd series from C_9 to C_{31}. The iso acids from C_{10} to C_{28} occur also, but only in the even series (*49*). The C_{15} and C_{17} (+)-anteiso and iso acids have also been found in the liver oil of the shark *Galeorhinus australis* (*17*). In the preen glands of ducks and geese levorotatory branched-chain acids occur in quantity as octadecyl esters. In the preen glands of ducks 4-methylhexanoic acid (*336, 337*) is the main fatty acid constituent, and in geese a dimethyl branched C_{14} acid predominates (*338*). The occurrence of considerable amounts of branched-chain acids in the sebum of various mammals has been reported, but the precise nature of these acids has not been revealed (*304, 305*). In the lipids of tubercle bacilli the branched-chain fatty acids are strikingly different from those already discussed. Here the lipids contain a large proportion of the high molecular weight (about C_{88}) mycolic acids with long alkyl side chains, lower molecular weight acids including 10-methylstearic (*50, 51*), (+)-2,4,6-trimethyltetracos-2-enoic (mycolipenic) (*52*) acid and (—)-2,4-6-trimethyloctacosanoic (mycoceranic) (*53*) acid.

The branched acids of the tubercle bacilli as well as those of certain other acid-fast bacteria are characterized by the fact that the side chains are located near the center of the chain or near the carboxyl group. It would appear that in this respect there is a relationship between the elaboration of specific fatty acid types and the position of the organism in the phylogenetic scale.

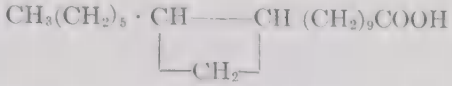
4. *Cyclic Acids*

In the acids possessing a cyclic structure the occurrence of C_{16} and C_{18} acids with a terminal cyclopentenyl ring is peculiar to the seed fats of many species belonging to the Flacourtiaceae, thereby affording an excellent chemical indicator for this family. Apart from these acids, the only other known lipid fatty acids possessing a cyclic structure are the cyclopropene acids, sterculic acid



$$\mathrm{CH_3(CH_2)_7}\underset{\mathrm{CH_2}}{\mathrm{C{=}C}}\cdot\mathrm{(CH_2)_7COOH}$$

found in *Sterculia foetida* (*55*) (Sterculiaceae) and a related C_{18} acid, malvalic acid, found in *Malva verticillata* and *M. parviflora* (*254*) (Malvaceae), as well as the cyclopropane acid—lactobacillic acid



$$CH_3(CH_2)_5 \cdot \underbrace{CH\text{———}CH}_{CH_2}(CH_2)_9COOH$$

found in *Lactobacilli* and *Phytomonas tumefaciens* (*206*).

The presence of cyclopropene acids in the seed fats from two species from different families may have little significance in the classification of plants. On the other hand, the elaboration of lactobacillic acid in the several species of lactobacilli examined may be significant in providing a biochemical marker that could assist in the identification of species in this group.

C. FATTY ACID COMPOSITION OF DEPOT FATS IN RELATION TO THE EVOLUTION OF ANIMALS

In plants the tendency toward the simplification in fatty acid composition, as we proceed from the less organized to the more highly organized forms of life, is not well defined.

In contrast, in the animal kingdom we find that the composition of the depot fats shows a marked simplification in passing from the fats of aquatic animals to those of land mammals (see Table III). The fats of amphibians and reptiles are intermediate in their fatty acid composition between land mammals and aquatic species. These changes in fatty acid composition have been related by Hilditch and Lovern (*140*) to evolutionary development. However, the fact that the fats of aquatic mammals and fishes have similar fatty acid composition, suggests that the environment rather than evolutionary development has been responsible for the formation of the aquatic type of fat. Indeed, the present author (*151*) regards the increasing simplicity in the composition of animal fats as one proceeds from the lower to the more highly organized forms of life as due mainly to the fortuitous influence of the diet and only in part to the animals themselves.

At first the organism, as exemplified by fishes, is apparently unable to make fat from the nonfatty (protein) constituents of the diet (*349*) and the dietary fat is usually deposited largely unchanged. In some instances, however, the polyethenoid acids are hydrogenated to give monoethenoid and saturated acids, or even partly converted to alcohols. An elongation or shortening of the carbon chain may also occur, and the fatty acids may be differentially distributed between the different depots [cf. Shorland (*89*)]. These modifications, while altering the nature of the fatty acids, still leave the unmistakable pattern of the aquatic type of fatty acid distribution.

In accordance with the views just expressed, changes in the composition of fish depot fats are produced with changes in the fatty constituents of the diet. Grass-fed carp, for example, deposit diene and triene acids characteristic of grass lipids, but which are not found elsewhere in aquatic life (*350*). Similarly, eels fed a diet of herring flesh modify their depot fats to resemble more closely those of herrings (*351*). In addition mullet (*Mugil cephalus*) when fed a "fat free" diet are to a large extent depleted of their reserves of polyunsaturated acids. On addition of menhaden oil to the diet, the polyunsaturated acids are deposited in the same proportions as fed. Similarly, in mullet fed a commercial cooking fat made from cottonseed oil, the depot fats again resemble the dietary fat (*352*).

In the next stage, represented by amphibians, reptiles, and nonruminants, the dietary carbohydrate and protein is used to produce endogenous fat. Such fat consists mainly of palmitic (about 27–30%) and oleic acids with lesser amounts of stearic, myristic, and palmitoleic acids. In addition, as in fishes, the dietary fats are also deposited so that the depot fats now consist of a mixture of endogenous and exogenous fats. The composition of the depot fat will now depend on the nature and amount of the dietary fat, and on the rate of growth, as suggested by Callow (*353*) provided the diet, as in coconut oil, does not contain lower fatty acids that are rapidly metabolized.

Finally, in ruminants the depot fats are formed largely from the acetate and lower volatile fatty acids produced in the rumen from carbohydrates and proteins which are broken down by the action of the microorganisms. The dietary fat appears to have little influence on the composition of the depot fat. This is because the dietary unsaturated fatty acids, such as linolenic acid, are hydrogenated to form saturated acids along with lesser amounts of monounsaturated and traces of diunsaturated acids, which are *trans* and positional isomers of oleic and linoleic acids (*118–121*).

D. FATTY ACIDS OF WAXES, PHOSPHOLIPIDS, AND RELATED SUBSTANCES AND THE CLASSIFICATION OF ORGANISMS

The depot fats of plants and animals have been shown to vary in the types and proportions of fatty acids. These variations may indicate some degree of differentiation in organisms in different parts of the phylogenetic scale. As disclosed in Section VII,C above, however, the differentiation exhibited in animal depot fats is, to a considerable extent, fortuitous and dependent on the dietary fat. In the depot fats of plants, including those of cryptogams and seed fats, the differentiation in fatty acid types and composition sometimes appears to be characteristic of the species, genus, or family.

The limited data on the fatty acids of plant waxes have not shown the same degree of differentiation as have the depot fats. Nevertheless the waxes of acid-fast bacteria, which consist of the high molecular weight mycolic acids combined with polysaccharides, contrast sharply with those of the rest of the plant and animal world. The waxes of higher plants and insects appear to have similar fatty acids, namely the C_{24} to C_{34} *n*-saturated acids with perhaps the corresponding ω-hydroxy acids (*23*). In the phanerogams, the seed lipids of *Simmondsia californica*, which are waxes consisting mainly of eicos-11-enoic acid combined with docosenol (*263*), form a remarkable variation from the other seed lipids, which are invariably glyceridic. The depot lipids of the sperm whale likewise provide an exception in the animal kingdom in consisting of waxes of unsaturated alcohols combined with C_{10}–C_{18} *n*-saturated and C_{14}–C_{22} unsaturated acids, as in the fats of marine fish.

The waxlike secretions of the skin (*304*, *305*, *334*), hair, and preen glands (*336–338*) afford a unique variety of branched, *n*-odd-numbered saturated and unsaturated fatty acids, the proportions and nature of which appear to vary from one species to another. As many of these acids are not found in the diet, the fatty acid composition could possibly be used to characterize the species.

The value of the fatty acids in waxes as a guide to the classification of species still remains to be explored.

The limited data on seed phospholipids show that their fatty acid composition is similar to that of the corresponding glycerides. In the animal kingdom, the phospholipids of aquatic and terrestrial species show no marked differences, the amounts of C_{20} and C_{22} highly unsaturated acids in both being considerable. As these acids are absent from land plant lipids, the fatty acid composition of phospholipids of land plants and animals shows a greater divergence than between the corresponding glycerides.

The sphingolipids are not found in plants and these, together with their characteristic fatty acids, including tetracosanoic, α-hydroxytetracosanoic, tetracos-15-enoic, and α-hydroxytetracos-15-enoic acids serve to differentiate animals from plants.

E. CONCLUDING REMARKS

If evolution applies to morphology, it could be expected to apply also to the chemical constitution of organisms. Gibbs (*354*) has recently summarized the attempts made by chemists and botanists to relate the chemical characteristics of plants to their classification. The results so far obtained have not provided a clear indication of biochemical evolution in plants, possibly because of the absence of adequate classification of biochemical substances. The question of discerning what is and what is not useful as a signpost in the direction of the evolutionary process is still far from decided.

Thus it is perhaps not surprising that in the field of lipids the types and distribution of fatty acids have not provided a simple means for the classification of organisms except in certain restricted parts of the animal and plant kingdoms. Nevertheless, there are found some connections between the types of fatty acids elaborated and biological classification. It is possible that a greater knowledge of the occurrence of fatty acid types in lipids could provide a more positive guide of wide application in the classification of plant and animal species, and permit correlations between the chemical make-up of organisms and their position on the phylogenetic scale.

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CHAPTER 2

Sterols: Their Structure and Distribution

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I. Introduction

The discovery of the organic nature of gallstones in the latter part of the eighteenth century marks the beginning of the study of the chemistry and biochemistry of the sterols. In 1816 Chevreul named the principal component of such stones "cholesterine" to connote that it is a fatty (*-ine*) solid (*stereos*) of bile (*chole*) (*1*). After Berthelot (*2*) had shown in 1859 that this compound is an alcohol, its name was changed to the more descriptive one of cholesterol in all but the German literature. The term sterol was subsequently used to embrace compounds closely related to cholesterol, and the term steroids encompasses the multitude of naturally occurring and synthetic compounds which may be viewed as derived from sterols. Thus a host of compounds of great physiological importance and of intriguing structural features has been combined under an unimaginative name the English equivalent of which is solids.

---

† Deceased.

By the middle of the nineteenth century cholesterol had been isolated from animal tissues and secretions, from bile fluid and blood, from egg yolk and brain, and by the end of the century its ubiquity in animals had been generally recognized. In gallstones and in brain tissues cholesterol is present in the free state almost exclusively. In other places it occurs mainly as fatty acid esters. Thus 73% of the cholesterol of blood serum, 0.15–0.25 gm. per 100 ml., is present in the esterified form (*3*).

After cholesterol had been isolated from animal sources too numerous to mention here, the belief took hold that it was indeed the universal sterol of the animal world. Even in a most recent monograph reference is made in the preface to "the universal distribution of cholesterol in all animal tissues." If it were true indeed that cholesterol is the principal sterol of all animals, there would be but little justification for the writing of a chapter which is to deal with the comparative biochemical aspects of sterols. Fortunately, however, there exists a significant diversity among the principal sterols of animals. This became apparent during the first decade of the present century with the discovery that in certain animals cholesterol is replaced totally or in part by closely related compounds. New sterols had been obtained from a sponge (*4*) and from insects (*5*). Dorée (*6*) was the first to point to the possible significance of variations among animal sterols. His study was one of the first comparative biochemical studies, and it merits the designation of a classic in its field. The object of his investigations was clearly outlined in the opening paragraph of his report: "If cholesterol is a body which is one of the primary constituents of animal protoplasm, we should expect to find it not only in the highly organized animals, but throughout the series from Chordata to Protozoa, or if cholesterol were not present, its place should be filled by other and closely related forms. In the latter case it might be found that each of the great classes of the animal kingdom was characterized by the presence of a different member of the cholesterol family. On the other hand, if cholesterol is not of primary importance to all forms of life, it is not impossible that animals might be found in the composition of whose protoplasm it did not enter."

Dorée isolated and characterized the sterols from one or two representatives of the larger phyla of the animal kingdom. Since he found sterols in the animals under investigation he inferred their presence in all animals. He obtained cholesterol not only from warm-blooded animals, but also from other vertebrates and from invertebrates including such primitive forms as sea anemones. While he did not find a sterol typical for each phylum, Dorée showed that in at least two invertebrates, a sponge and the common starfish, cholesterol is replaced by another related substance. His studies were paralleled at about the same time, and for the same reasons, by those of Welsch (7). They were published, however, in form of a dissertation only, and they consequently never received quite the attention they deserve. As early as fifty years ago, therefore, sufficient evidence had been accumulated to demonstrate clearly that there exists a diversity of sterols, at least among the invertebrates.

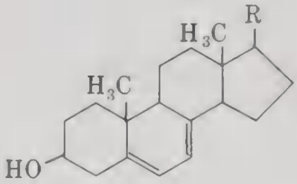
For twenty-five years thereafter the field of comparative biochemistry of sterols remained dormant. During this period numerous papers appeared with incidental references to the occurrence of cholesterol in vertebrates, merely adding further testimony to the ubiquity of this sterol in that phylum. Occasional references to the sterols of invertebrates were often of little value because of the *a priori* assumption that cholesterol is the sterol of all animals. Only after the elucidation of the structural skeleton of cholesterol in 1932 had the time become ripe for a resumption of studies which had had such a promising beginning. Due to the efforts of groups of investigators chiefly located in the United States and Japan, data had become available by 1949 to permit the author to survey this field from the viewpoint of comparative biochemistry, to point toward some interesting connections between the sterol content of an animal and its classification, and to speculate on the evolutionary aspects of the sterols (8).

II. The Sterols

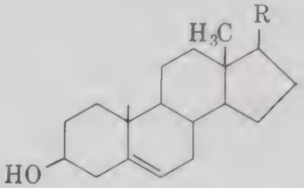
A. General Properties

Sterols are admirably suited for comparative biochemical research. They appear to be present in all animals and in a diversity so desirable for comparative studies. In addition, they offer considerable practical advantages. They are remarkably stable compounds particularly when embedded in other organic material. Only the $\Delta^{5,7}$-sterols (I), minor components of many sterol mixtures, are prone to undergo air oxidation. The relative stability of other sterols permits the collection and storage of biological material, air-dried or preserved by liquids, without fear of substantial alterations of its sterol content. It also makes possible the utilization of carefully selected museum specimens occasionally needed to complete a comparative study. Sterols have also been isolated from soils, and there exists good evidence for their fossil occurrence (9).

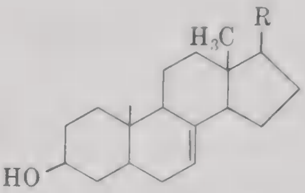
The isolation of sterols from biological material is generally accomplished with ease. Recrystallization of the unsaponifiable fraction of the fat is frequently sufficient to obtain a crystalline sterol mixture, free from other alcohols and from hydrocarbons. Where sterols constitute only a minor fraction of the unsaponifiable matter, removal of the non-



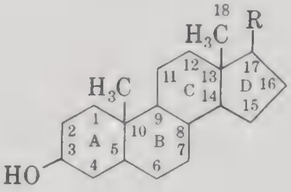
(I)
$[\alpha]_D -90^\circ$



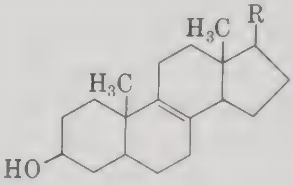
(II)
$[\alpha]_D -25^\circ$ to -70°



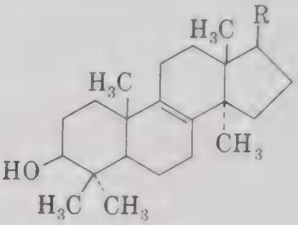
(III)
$[\alpha]_D -20^\circ$ to $+10^\circ$



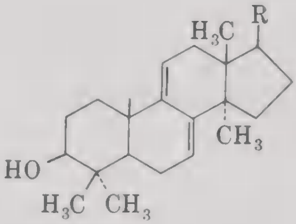
(IV)
$[\alpha]_D +15^\circ$ to $+30^\circ$



(V)
$[\alpha]_D +30^\circ$ to $+50^\circ$



(VI)
$[\alpha]_D +60^\circ$



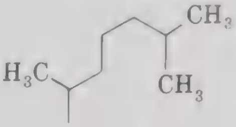
(VII)
$[\alpha]_D +70^\circ$ to $+80^\circ$

sterol material may be achieved by vacuum distillation or chromatographic separation. The sterols may be precipitated selectively and quantitatively by digitonin. This reagent not only permits quantitative comparative studies but also the easy isolation of sterols from relatively small amounts of biological source material. The recovery of the sterols from the digitonides is best accomplished by Bergmann's variation of Schoenheimer's procedure (*10*).

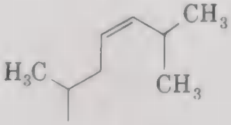
In contrast to the ease of isolation of the sterol mixtures are the difficulties often encountered in the separation of their components. The partition of sterols differing in the number and location of double bonds may be achieved by the chromatography of their esters, among which the colored *p*-azoylbenzoates (*11*, *12*) and the radioactive *p*-iodobenzoates-I^{131} have been found particularly useful (*13*). The radioactive esters lend themselves well for the delicate separation of small amounts of material. Frequently, however, sterol mixtures contain homologs differing only by a methyl or ethyl group in either one of the two epimeric positions on the 24-carbon atom of the side chain (E, G; F, H; J, L; and K, M). It is the satisfactory separation of such sterols which still remains one of the most annoying obstacles to the progress of comparative biochemistry of sterols. It now appears, however, that refinement of vapor phase chromatography with its extension to compounds of higher molecular weight will make possible a speedy and near-quantitative separation of sterols inclusive of homologs.

Great strides have been made in recent years in the identification of sterols and the elucidation of their structures. Since the days, one hundred years ago, when an empirical formula for cholesterol was first proposed, the determination of the molecular size of a sterol and the number of its carbon atoms has always been a major problem. Conventional carbon-hydrogen analyses of the sterols and their usual derivatives are useless in differentiating between cholesterol and its homologs. This so far has been possible only through careful analyses of special derivatives such as the dinitrobenzoates of sterols, or their di- and tetrabromides, or by quantitative saponification of their acetates (*14*). It has now been shown by Jones and his associates (*15*) that the size of a sterol side chain may be determined on a 30-mg. sample by an ingeniously simple method. The sterols are heated with an acid catalyst to 400°, when the side chain breaks off. The volatile products of this cracking reaction are collected and subjected to vapor-phase chromatographic analysis. The fraction representing the side chains of C_{27}-, C_{28}- or C_{29}-sterols may be readily differentiated. This method which as yet is restricted to sterols with unsaturated side chains may be expected to replace all the older methods for determining the molecular size of a sterol.

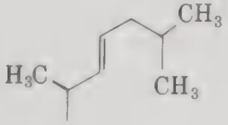
One of the first practical applications of ultraviolet absorption



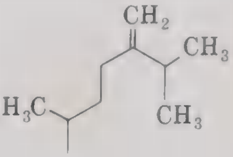
A
C_{27}-series



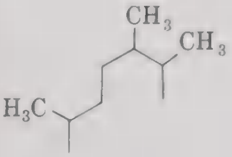
B
C_{27}-series



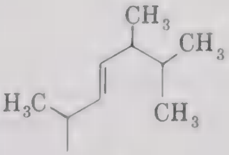
C
C_{27}-series



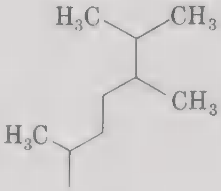
D
C_{28}-series



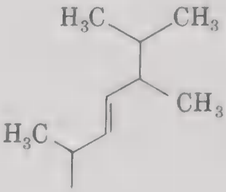
E
24-α-series



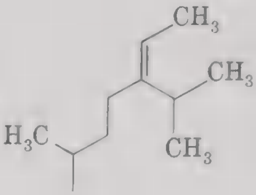
F
24-α-series



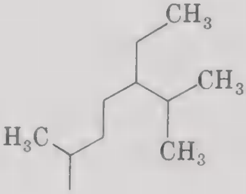
G
24-β-series



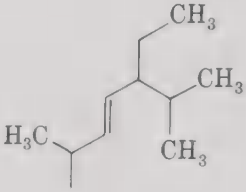
H
24-β-series



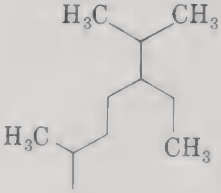
I
C$_{29}$-series



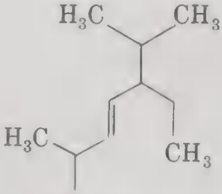
J
24-α-series



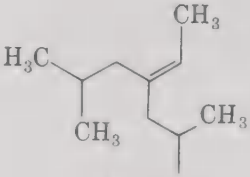
K
24-α-series



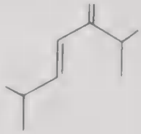
L
24-β-series



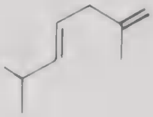
M
24-β-series



N
20-iso or 20-β-series



O



P

Diunsaturated side chains

spectroscopy was in the field of sterols. More than thirty years ago it served to discover that the precursors of vitamin D in sterol mixtures are compounds with conjugated double bonds. It is still the best method for the facile detection of such groups in sterols. Since they are comparatively rare, however, the usefulness of ultraviolet absorption spectra in the field of natural sterols is rather limited. In the meantime the infrared spectra of sterols have become an indispensable tool in the identification of sterols and the elucidation of their structures. Prior to the introduction of infrared spectroscopy, for example, a terminal methylene group, $CH_2=$, in the side chain could be detected only by ozonolysis, a method wasteful of precious material. Today its presence is readily recognized by its absorption bands in the infrared spectrum (see below). The same holds true for many other double bonds in the molecule. A particularly lucid treatment of the significance and practical application of ultraviolet absorption spectroscopy is found in Fieser and Fieser's excellent monograph on steroids (*14*), and that of infrared spectroscopy in Cole's informative review (*16*).

At the time of the discovery of most of the natural sterols not only were their structures unknown, but often also their correct empirical formulas and the degree of their uniformity. To facilitate discussion of the occurrence of such sterols, they were generally given names relating them to the organism from which they had first been derived. These are names of convenience, and any confusion they will cause will be but temporary. Once the structures of such sterols have been fully established, such names should be discarded and be replaced by others denoting their chemical structure and their relation to such well-known sterols as cholesterol. Long-accepted names will probably always be retained. Thus it appears unlikely that the short name ergosterol will be replaced by the more instructive but rather cumbersome 3β-hydroxy-24α-methyl-$\Delta^{5,7,22}$-cholestatriene or even the shorter 24α-methyl-7,22-bisdehydrocholesterol. The host of other sterols substituted by alkyl groups at C-24 are best referred to as derivatives of cholesterol as was suggested by the author some time ago (*17*). The organic chemist knows that the occasional, Babylonian confusion of names for natural products will never be of long duration and will terminate once the structures of the compounds have been ascertained. In contrast, no end appears to be in sight to the exasperating confusion caused by the multiplicity of names for biological species and the lack of agreement in their application.

B. STRUCTURES

Until a few years ago the term sterols embraced only those steroids that differ from cholesterol only in the number and position of double bonds in the rings or the side chain, or the attachment to the side chain of substituents such as alkyl groups at C-24. With the recognition of the structural and biosynthetic connections between sterols and triterpenoids such "methylsterols" as lanosterol and agnosterol (VI and VII; $R = B$) must also be included among the natural sterols. At present, however, very little is known about the occurrence of methylsterols in animal tissues or products other than wool grease (lanolin).

The sterols which are known or suspected to occur in animals may conveniently be divided into several groups on the basis of the degree and location of unsaturation in their ring structures (*18*). Such unsaturation significantly influences the direction and magnitude of the optical rotatory power of the sterols, thereby permitting their preliminary classification. Very pronounced, negative specific rotations, in excess of —90°, are characteristic of the presence in ring B of a $\Delta^{5,7}$-system of conjugated double bonds (I). A more moderated levorotation of —30 to —45° is typical of sterols with a double bond in the 5,6-position (II), and rotations of —50 to —70° point to the presence of additional unsaturation in the side chain. Sterols with a double bond in the 7,8-position (III) show specific rotations rather close to zero, or somewhat more negative when accompanied by side-chain unsaturation. Absence of unsaturation in the ring systems (IV) is revealed by a significant, positive specific rotation from +10 to +30°, and more pronounced dextrorotations, of +40 to +50° point to the presence of a double bond at 8,9 (V). Higher positive rotations generally indicate the presence of triterpenoids which are not included among the sterols with the exception of such methylsterols as (VI) and (VII).

Additional differences between the sterols are associated with the side chain, its unsaturation and its size. In many invertebrate sterols and nearly all plant sterols the eight-carbon side chain of cholesterol is replaced by a nine-carbon unit with a methyl branch at C-24 (E–H), or a ten-carbon unit with an ethyl group at this position (J–M). Such substitution confers asymmetry upon C-24, and hence two sets of isomers, epimeric at this position, are expected to exist. Although nature is rather stereospecific and generally synthesizes but one of the two possible isomers, in this case natural representatives of both epimeric series have become known. In several instances their absolute configurations have been rigidly established. Following a proposal made by Fieser in the forthcoming new edition of his well-known monograph on sterols (*14*), a methyl or ethyl group at C-24 will receive the designation α when on the same side of side chain as the α-oriented methyl group at C-20 (E, F and J, K), and the designation β, when in the opposite epimeric position (G, H and L, M).

The most common unsaturation in the side chain is the double bond at 22,23. As yet it has been found only in sterols with alkyl substituents at C-24. In all known examples this double bond is *trans*-oriented, as may be readily recognized by the characteristic peak at 10.30 μ in the infrared spectrum (*16*). The double bond noticeably enhances the levorotatory power of a sterol. A sterol unsaturated at 24,25 (II, R = B) is thought to represent an intermediate step in the biosynthesis of cholesterol. In animal sterols this side chain (B) has so far been found only in conjunction with a Δ^5-sterol ring system (II) and among the methyl sterols (VI and VII). Unsaturation has also been found between C-24 and the group attached to it (D and I). The C-24-methylene sterols have long been suspected to be present among the minor yeast sterols (III, V, R = D). 24-Methylenecholesterol (II, R = D) enjoys a rather wide distribution among marine invertebrates. The presence of this terminal methylene group may readily be recognized by its characteristic bands at 11.31 μ in the infrared spectrum. The 24-ethylidene side chain (I) has so far been found only in conjunction with the Δ^5-ring system as in the algal sterols. The 24-methylene (D) and 24-ethylidene (I) side chains may well occupy pivotal positions. Their biochemical hydrogenation establishes asymmetry at C-24 and could thus lead into the α-series (E, F and J, K) or β-series (G, H and L, M) of 24-substituted sterols (*8*).

There are some indications that sterols with di-unsaturated side chains such as O and P may also be encountered in animal tissues. Side chain O, a conjugated system quite prone to undergo air-oxidation has already been found among the minor yeast sterols (I, R = O) (*19*). The other, P, is suspected to occur in a highly unsaturated fraction of certain mollusk sterols (*20*). The methyl-substituted C-20 is also asymmetric, and the natural occurrence of two sets of isomers epimeric at this point has for some time been suspected (*21*). The vast majority of known sterols possess at this point the same configuration as cholesterol (II, R = A). Rather recently, however, it has been found that sargasterol, the principal sterol of *Sargassum* seaweed, is the C-20-epimer of fucosterol (II, R = N) (*22*). It is probable that additional members of this 20-iso series will be encountered among the many, as yet unseparated, mixtures of animal sterols now in the hands of investigators.

C. Types of Natural Sterols

The following paragraphs list in their appropriate groups the sterols to be encountered in animals. Several sterols have been included which as yet have been isolated only from plants but the occurrence of which in animals is suspected for reasons of analogy. With but few exceptions the listing is restricted to sterols the structures of which have been well established either by methods of degradation or synthesis. Because of the difficulties encountered in obtaining truly pure sterol samples, the reported physical properties of the sterols and their acetates are subject to changes as purer samples become available. Ultimately only synthetic materials will be used as reference samples. As yet, however, the syntheses of the sterols most difficult to obtain pure, 24-alkyl cholesterols of known configuration, have not been accomplished. The names and properties of sterols of uncertain structure or uniformity will be given in connection with general discussions of the sterols of the phyla or classes of animals from which they have been obtained.

1. *Group I (Structure I): $\Delta^{5,7}$-Sterols or 7-Dehydrosterols with Specific Rotations in Excess of $[\alpha]_D$ −90°* [*]

The most characteristic structural feature of the sterols belonging to this group is the system of conjugated double bonds in ring B. It not only accounts for their highly negative rotation, but also for their very characteristic ultraviolet absorption spectrum. It sets these sterols apart from all others which do not absorb light in this region. It also makes possible the detection and quantitative determination of very small amounts of $\Delta^{5,7}$-sterols in mixtures. This spectrographic evidence, however, detects only the presence of conjugation in ring B and no other structural features such as the size of the side chain. This should be borne in mind when reviewing the older literature, which contains many references to the occurrence of ergosterol or 7-dehydrocholesterol in mixtures, when the less specific term $\Delta^{5,7}$-sterol would have been more accurate. Relatively large quantities of sterols of type I with unknown side chains are found in mollusks. They will be discussed in the chapter dealing with the sterols of bivalves.

A conjugated system of double bonds is very reactive and prone to undergo rearrangements and other reactions induced by physical and chemical means. Of greatest biological significance is the action of ultraviolet light on $\Delta^{5,7}$-sterols which leads through a series of interesting rearrangements to the vitamins D. It is for this reason that $\Delta^{5,7}$-sterols have been referred to as provitamins D, although they may not all acquire antirachitic activity upon irradiation. In another light reaction, in the presence of air and a photosensitizer $\Delta^{5,7}$-sterols absorb oxygen to form nicely crystalline and rather stable peroxides (*14*).

7-Dehydrocholesterol (provitamin D_3) (I, R = A), $C_{27}H_{42}O$, m.p. 150°, $[\alpha]_D$ −114°; acetate, m.p. 130°, $[\alpha]_D$ −85°. It is probably the most widely distributed provitamin D of the animal world. While it normally accompanies cholesterol or other sterols in but small amounts, it may in certain cases constitute as high as 27% of the sterol mixture (see under Mollusks, Section VIII). 7-Dehydrocholesterol is technically prepared from cholesterol for the manufacture of vitamin D_3, of which large quantities are needed in poultry raising.

24α-Methyl-7-dehydrocholesterol (22,23-dihydroergosterol; provitamin D_4) (I, R = E), $C_{28}H_{46}O$, m.p. 153°, $[\alpha]_D$ −109°; acetate, m.p. 158°, $[\alpha]_D$ −75°. It has only been obtained by a selective hydrogenation of the side chain of ergosterol (II, R = F) (*23*). Like its 24β-epimer, described below, it is a probable component of the provitamin D mixtures of invertebrates. Upon irradiation it affords vitamin D_4 (*24*).

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[*] Unless stated otherwise, the rotations were taken in chloroform.

24β-Methyl-7-dehydrocholesterol (7-dehydrocampesterol) (I, R = G), $C_{28}H_{46}O$, m.p. 165°, $[\alpha]_D$ −109°; benzoate, m.p. 157°, clear at 164°, $[\alpha]_D$ −50°. It has been prepared by standard reactions from campesterol (II, R = G) (*25*). In contrast to its 24α-epimer it acquires only feeble antirachitic activity upon irradiation (*25*). The configuration of the 24-methyl group is therefore of some significance to vitamin D activity.

Ergosterol (provitamin D_2) (I, R = F), $C_{28}H_{44}O$, m.p. 168°, $[\alpha]_D$ −132°, infrared spectrum (*26*); acetate, m.p. 181°, $[\alpha]_D$ −90°. It is appropriately regarded as the most typical sterol of fungi. Its inclusion among animal sterols is justified since it has been found as a major component of the sterol mixture from a snail and possibly also other mollusks (see Section VIII).

24α-Ethyl-7-dehydrocholesterol (7-dehydro-β-sitosterol) (I, R = J), $C_{29}H_{48}O$, m.p. 144–145°, $[\alpha]_D$ −116°; acetate, m.p. 151–152° (*27, 28*) $[\alpha]_D$ −71°, and *24β-ethyl-7-dehydrocholesterol* (7-dehydro-γ-sitosterol (?); 7-dehydroclionasterol (*29*), (I, R = L), $C_{29}H_{48}O$, m.p. 138°, $[\alpha]_D$ −98°; acetate, m.p. 140°, $[\alpha]_D$ −72°. Both sterols have been prepared by standard reactions from starting materials of questionable uniformity and hence cannot be regarded with certainty as pure substances. Their occurrences in provitamin D mixtures has been suspected (see Section VIII).

7-Dehydrostigmasterol [24α-ethyl-7,22-bisdehydrocholesterol; corbisterol (?)], (I, R = K), m.p. 154°, $[\alpha]_D$ −113°; acetate, m.p. 172°. It was first prepared from stigmasterol (II, R = K) (*30*), and it has been suggested to be identical with corbisterol from mollusks (see Section VIII).

2. *Group II* (*Structure II*): *Δ^5-Sterols with Specific Rotations of $[\alpha]_D$ −25° to −70°*

This group includes the sterols most commonly encountered in animals and plants. Whether a sterol is Δ^5-unsaturated and of a reasonable degree of purity may be learned from a comparison of its molecular rotation with those of some of its derivatives. According to the rules of rotational shift, conversion of all sterols of group II to their acetates or benzoates should be accompanied by analogous shifts in molecular rotations. Barton (*31*) found that the acetate shift of Δ^5-sterols is -35 ± 10 and the benzoate shift $+81 \pm 10$. A newly found, levorotatory sterol may safely be regarded as belonging to group II when the rotational shifts of its acetate and benzoate are close to the known values. Inversely, sterols suspected to be Δ^5-unsaturated but with molecular rotation differences at variance with such values must be regarded as lacking uniformity or belonging to another group.

Cholesterol (II, R = A), $C_{27}H_{46}O$, m.p. 149.5–150°, $[\alpha]_D$ —39.5°, infrared spectrum (*26*); acetate, m.p. 114–115°, $[\alpha]_D$ —47.5°. It is the best known and most readily available of all sterols. It is the typical sterol of the vertebrates and has also been found in many invertebrates including protozoa and sponges (Sections III and IV). More recently it has been isolated in significant quantities from a number of red algae (*32*). This significant discovery has removed the last reason for the artificial division of sterols into zoosterols, phytosterols, and mycosterols which had so long been in use.

24-Dehydrocholesterol (desmosterol) (II, R = B), $C_{27}H_{44}O$, m.p. 117° (*33*), 120–121° (*34*); λ_{max} 2.96, 7.28, 12.51 μ in KBr; $[\alpha]_D$ —40°; acetate, m.p. 92–93° (*34*), 101° (*33*), $[\alpha]_D$ —41°. It is believed to represent one of the final steps in the biosynthesis of cholesterol. It has been isolated in small amounts from chicken embryos (*35*) and in larger quantities from barnacle sterols (*33*). The structure has been established by degradation and synthesis (*33, 34*).

22-Dehydrocholesterol (II, R = C), $C_{27}H_{44}O$, m.p. 135°, $[\alpha]_D$ —57°, λ_{max} 2.97, 7.29, 7.34, 10.29, and 12.51 μ in KBr; acetate, m.p. 126°, $[\alpha]_D$ —63°. This sterol which as yet has become known only as synthetic material (*36*) carries a side chain analogous in its unsaturation to those found in many natural, 24-alkyl-substituted sterols (F, H, K, and M). It has long been suspected to occur in some of the more complex sterol mixtures. Its physical properties are so similar to those of β-sitosterol and of many poorly defined sterol mixtures that it may easily be missed.

24-Methylenecholesterol (chalinasterol) (II, R = D), $C_{28}H_{46}O$, m.p. 142° (*37*), 145–146° (*38*); $[\alpha]_D$ —35° (*37*), —42° (*38*), infrared spectrum (*38*); acetate, m.p. 135–136°, $[\alpha]_D$ —42 to —47°. It appears to be quite widely distributed among many invertebrates such as sea anemones, sponges, and bivalves. Before its structure had first been established by Idler (*37*) it had been described under a variety of names which will be discussed under mollusk sterols. The sterol has been synthesized (*38, 39*).

24α-Methylcholesterol (22,23-dihydrobrassicasterol) (II, R = E), $C_{28}H_{48}O$, m.p. 158°, $[\alpha]_D$ —46°; acetate, m.p. 145°, $[\alpha]_D$ —46°. As yet only obtained by selective hydrogenation of brassicasterol (II, R = F) (*40*), this sterol may be assumed to occur in some of the unresolved sterol mixtures from bivalves.

24β-Methylcholesterol (campesterol) (*41*) (II, R = G), $C_{28}H_{48}O$, m.p. 158°, $[\alpha]_D$ —33°; acetate, m.p. 138°, $[\alpha]_D$ —35°. Although this sterol has with certainty been isolated only from plants, its structural relationship to other sterols found in invertebrates makes its occurrence in animals quite probable.

24α-Methyl-22-dehydrocholesterol (brassicasterol) (II, R = F), $C_{28}H_{46}O$, m.p. 148°, $[\alpha]_D$ −64°; acetate, m.p. 157° $[\alpha]_D$ −65°. First obtained from rapeseed oil, this sterol has been isolated from several invertebrates, among which it appears to be fairly widely distributed.

24β-Methyl-22-dehydrocholesterol (II, R = H). A sterol, chalinasterol, which had originally been assigned this structure has been shown to be 24-methylenecholesterol (II, R = D) (*38*). There seems to be little doubt that a sterol of structure (II, R = H) is present in some of the more complex sterol mixtures. Its isolation in a reasonable state of purity, however, has not yet been accomplished.

24α-Ethylcholesterol (β-sitosterol) (II, R = J), $C_{29}H_{50}O$, m.p. 137°, $[\alpha]_D$ −37°, infrared spectrum (*26*); acetate, m.p. 127° $[\alpha]_D$ −42°. Widely distributed among plants and described under a variety of names (*18*) this sterol generally occurs in mixtures from which it can be separated only with great difficulties. It is generally accompanied by its 24β-epimer. A reliable reference sample is best prepared by selective hydrogenation of stigmasterol (II, R = K) (*42*). Its occurrence in mollusks has been reported.

24β-Ethylcholesterol [γ-sitosterol; clionasterol (?)] (II, R = L), $C_{29}H_{50}O$, m.p. 148°, $[\alpha]_D$ −43°; acetate, m.p. 144°, $[\alpha]_D$ −45°. In plants, this sterol is found mainly mixed with its 24α-epimer as minor or major component. It is difficult to obtain pure. The same holds true for clionasterol, which is widely distributed among invertebrates and which may well be identical with γ-sitosterol and possess structure (II, R = L) (*17*).

Stigmasterol (24α-ethyl-22-dehydrocholesterol) (II, R = K), $C_{29}H_{48}O$, m.p. 170°, $[\alpha]_D$ −46°, infrared spectrum (*26*); acetate, m.p. 144°, $[\alpha]_D$ −49°. Like other sterols first known from plants it also appears to occur in invertebrate sterol mixtures.

24β-Ethyl-22-dehydrocholesterol (poriferasterol) (II, R = M), $C_{29}H_{48}O$, m.p, 156°, $[\alpha]_D$ −49°, infrared spectrum (*26*); acetate, m.p. 147°, $[\alpha]_D$ −53°. This 24-epimer of stigmasterol is readily separated through its acetate tetrabromides. It appears to be widely distributed among marine invertebrates.

24-Ethylidenecholesterol (fucosterol) (II, R = I), $C_{29}H_{48}O$, m.p. 124°, $[\alpha]_D$ −40°, infrared spectrum (*26*); acetate, m.p. 118°, $[\alpha]_D$ −45°. This typical sterol of the brown algae, Phaeophyceae (*18*), has not yet been found in animals. It may be regarded as an intermediate in the biosynthesis of sterols of the types (II, R = J) and (II, R = L). The methyl group of the C-24 branch has been found to be *cis*-oriented with respect to the isopropyl group. A synthetic product with a *trans* orientation at this point is also known (*43*). *Sargasterol* (20-isofucosterol), (II, R = N), $C_{29}H_{48}O$, m.p. 132–133.5°, $[\alpha]_D$ −47.5°; acetate, m.p. 138–139°, $[\alpha]_D$ −53°. This sterol, as yet isolated only from *Sargassum* seaweed, has been assigned an isoconfiguration at C-20 on the basis of degradative (*22*) and synthetic evidence (*44*). It is believed that a similar isomerism occurs in other sterols. Since their structures have not definitely been established they will be dealt with under the animals from which they have been derived.

3. *Group III (Structure III): Δ^7-Sterols with Specific Rotations of $[\alpha]_D$ −20° to +10°*

The Δ^7-sterols, formerly also called "γ"-sterols belong to the most readily recognizable sterols. Without additional unsaturation their specific rotations are near zero, but more negative with a second double bond in the side chain. The differences between the molecular rotations of the sterols and their acetates and their benzoates are -15 ± 15 and $+20 \pm 14$, respectively (*31*). These sterols are relatively easily separated from sterol mixtures by chromatography of their azoyl esters (*11*). Small quantities of Δ^7-sterols in mixtures may be detected and estimated by means of colorimetric methods. One of them makes use of a modified Schoenheimer and Sperry reagent (*11*), and the other of the reduction of the colorless selenious acid to colored selenium (*45*).

Δ^7-Cholestenol (lathosterol) (III, R = A), $C_{27}H_{46}O$, m.p. 126°, $[\alpha]_D$ +6°, infrared spectrum (*26*); acetate, m.p. 119°, $[\alpha]_D$ +2°. After it had been known for some time as a synthetic product, this sterol was first found in nature as a companion of cholesterol in commercial preparations (*45*). It was subsequently isolated from the skin of animals and was found in particularly high concentrations in the skin sterols of rodents (*11*). Δ^7-Cholestenol is the principal sterol of certain primitive mollusks, and it is believed to be identical with hitodesterol from certain starfish sterol mixtures. It has also been shown to function as a precursor of cholesterol (*46*).

Δ^7-Ergostenol (fungisterol; 24α-methyl-Δ^7-cholestenol) (III, R = E), $C_{28}H_{48}O$, m.p. 146°; $[\alpha]_D$ 0°; acetate, m.p. 160°; $[\alpha]_D$ −5°; and *5,6-dihydroergosterol* (α-dihydroergosterol; 24α-methyl-$\Delta^{7,22}$-cholestadienol) (III, R = F), $C_{28}H_{46}O$, m.p. 176°, $[\alpha]_D$ −19°; acetate, m.p. 180°, $[\alpha]_D$ −20°. Both sterols are minor components of yeast sterol mixtures. It is probable that they also accompany their 24β-methyl epimers (*vide infra*) in starfish sterol mixtures.

24β-Methyl-Δ^7-cholestenol (stellastenol) (III, R = G), and *24β-*

methyl-$\Delta^{7,22}$-cholestadienol (stellasterol) (III, R = H) are believed to be some of the major sterols of starfish. They have not yet been isolated in a uniform state (see under Echinoderms, Section IX).

24-Methylene-Δ^{7}-cholestenol (episterol) (III, R = D), $C_{28}H_{46}O$, m.p. 151°, $[\alpha]_D$ $-50°$; acetate, m.p. 162°, $[\alpha]_D$ $-4°$. The structure of this compound, which is one of the minor yeast sterols, has been derived from degradation studies (*47*) and optical rotations (*31*); it needs confirmation by synthesis. This sterol may be viewed as an analog of 24-methylenecholesterol. As this is found among the Δ^5-sterol mixtures of marine invertebrates, so will episterol probably be encountered in corresponding Δ^7-sterol mixtures.

24α-Ethyl-Δ^{7}-cholestenol (Δ^7-stigmastenol) (III, R = J), $C_{29}H_{50}O$, m.p. 145°; $[\alpha]_D$ $+8°$; acetate, m.p. 157°, $[\alpha]_D$ $+7°$; and *24α-ethyl-$\Delta^{7,22}$-cholestadienol* (α-spinasterol) (III, R = K), $C_{29}H_{48}O$, m.p. 174°, $[\alpha]_D$ $-3°$, infrared spectrum (*26*); acetate, m.p. 187°, $[\alpha]_D$ $-5°$. They are the principal Δ^7-sterols of plants and may well occur in the complex Δ^7-sterol mixtures from certain marine invertebrates, such as starfish.

24α-Ethyl-$\Delta^{7,22}$-cholestadienol (chondrillasterol) (III, R = M), $C_{29}H_{48}O$, m.p. 169°, $[\alpha]_D$ $-2°$, infrared spectrum (*26*); acetate, m.p. 175°, $[\alpha]_D$ $-1°$. This sterol has been isolated from a sponge and an alga (*48*).

4. *Group IV (Structure IV): Sterols Saturated in the Ring System with Specific Rotations of $[\alpha]_D$ $+15°$ to $+30°$*

All known sterols belonging to this group are completely saturated with the exception of neospongosterol (IV, R = H) which carries a double bond in the side chain. They may therefore readily be distinguished from sterols of other groups by their inertness toward bromine and other double-bond reagents. When quite pure such sterols do not give any of the typical color reactions. Saturated sterols may be freed from unsaturated impurities by treatment with acetic anhydride and concentrated sulfuric acid in carbon tetrachloride. In this reaction only the unsaturated impurities form colored, acidic products, separable from the unreacted saturated sterols by alkali (*49*). The differences between the molecular rotations of ring-saturated sterols and of their acetates and benzoates are -34 ± 11 and $+2 \pm 3$, respectively (*31*). The last value is particularly characteristic and useful in gauging the uniformity of a saturated sterol.

Only in certain sponges have saturated sterols so far been found to be the principal components of sterol mixtures. In other cases they often represent such a small fraction of the mixture that they are easily overlooked. It is probable that eventually the saturated derivatives of all known sterols will be isolated from natural sources.

Cholestanol (dihydrocholesterol; β-cholestanol) (IV, R = A), $C_{27}H_{48}O$, m.p. 142°, $[\alpha]_D$ +24°, infrared spectrum (*26*); acetate, m.p. 111°, $[\alpha]_D$ +13.5°. It is a companion, 1–2%, of cholesterol in nearly all commercial preparations. In sterol mixtures from sponges it may occur in quantities well in excess of 50%.

24β-Methyl-Δ^{22}-cholestenol (neospongosterol) (IV, R = H), m.p. 150°, $[\alpha]_D$ +10°; acetate, m.p. 126°, $[\alpha]_D$ +8°. It is the only natural sterol now known which is unsaturated in the side chain only (*50*). It has been isolated from the spongosterol mixture of certain sponges.

The following saturated sterols have either been isolated from plant sterol mixtures or obtained by catalytic hydrogenation of other natural sterols. They are included because of their probable occurrence as minor components in sterol mixtures from invertebrates.

24α-Methylcholestanol (ergostanol) (IV, R = E), $C_{28}H_{50}O$, m.p. 145°, $[\alpha]_D$ +16°, infrared spectrum (*26*); acetate, m.p. 145°, $[\alpha]_D$ +6°.

24β-Methylcholestanol (campestanol) (IV, R = G), $C_{28}H_{50}O$, m.p. 147°, $[\alpha]_D$ +31°; acetate, m.p. 144°, $[\alpha]_D$ +18°.

24α-Ethylcholestanol (stigmastanol; β-sitostanol; dihydro-β-sitosterol) (IV, R = J), $C_{29}H_{52}O$, m.p. 138°, $[\alpha]_D$ +25°; acetate, m.p. 132°, $[\alpha]_D$ +25°.

24β-Ethylcholestanol (γ-sitostanol; dihydro-γ-sitosterol; poriferastanol) (IV, R = L), $C_{29}H_{52}O$, m.p. 144°, $[\alpha]_D$ +19°; acetate, m.p. 144°, $[\alpha]_D$ +10.

5. *Group V (Structure V): $\Delta^{8(9)}$-Sterols with Specific Rotations of +30° to +50°*

As yet only three natural representatives of this group have become known, and the structure of only one of them has been established with certainty. All of them are components of the mixtures of minor yeast sterols left after the removal of ergosterol. The $\Delta^{8(9)}$-sterols, which also have been called "δ"-sterols resemble the Δ^7-sterols in many of their reactions, but they differ from them in their pronounced dextrorotation. The molecular rotation differences between the sterols and their acetates and benzoates are −46.5 and +11.5, respectively (*31*). Renewed interest in these sterols has grown out of the realization that sterols of type V may constitute intermediate steps in the biosynthesis of other sterols. It is probable that a thorough search will reveal their presence in many sterol mixtures.

Zymosterol ($\Delta^{8(9),24}$-cholestadienol) (V, R = B), $C_{27}H_{44}O$, m.p. 110°, $[\alpha]_D$ +49°; acetate, m.p. 108°, $[\alpha]_D$ +35°. This sterol, first discovered as one of the most common components of the minor yeast sterol mixtures has assumed considerable importance in the discussions and experimentations on the final steps of the biosynthesis of cholesterol (VIII–XIII). It may be converted by rat liver homogenates into cholesterol (*51*) and it is believed to be present in trace amounts in pig liver (*52*).

24-Methylene-$\Delta^{8(9)}$-cholestenol (fecosterol) (V, R = D), $C_{28}H_{56}O$, m.p. 162°, $[\alpha]_D$ +42°; acetate, m.p. 159–161°, $[\alpha]_D$ +20°. The structure of this minor yeast sterol is based on some experimental evidence (*47*) and on molecular rotation differences (*31*). It needs verification by synthesis before it can finally be accepted.

6. *Group VI (Structures VI and VII): Trimethylsterols with Specific Rotations in Excess of $[\alpha]_D$ +50°*

These compounds, which contain six isoprene units joined in accordance with the isoprene rule, are of the class of triterpenoids. Their inclusion among the sterols is based on good chemical and biological evidence. They possess the typical sterol ring structure and a side chain common to other sterols, and differ from them only in the presence of three additional methyl groups. Cholesterol has been converted by chemical means into lanosterol, the best known of the trimethylsterols (*53*). The reverse has been carried out biologically in liver homogenates (*54*). In the biosynthesis of cholesterol (VIII–XIII), lanosterol (IX) has been shown to occupy a pivotal position (*46*) as the final product of the cyclization of squalene (VIII).

Trimethylsterols may be readily differentiated from other sterols by the molecular rotation differences between the sterols and their acetates. In contrast to those of the unmethylated sterols they are of positive direction, +17 ± 5 for type VI and +130 ± 10 for type VII. Table I lists the four trimethylsterols which have been isolated from wool fat. The most common of them, lanosterol, has been shown to be identical with cryptosterol, one of the minor components of yeast sterol mixtures (*47*).

TABLE I

TRIMETHYLSTEROLS

			Sterol		Acetate	
Name	Formula	Structure	M.p. (°C)	D°	M.p. (°C)	D°
Dihydrolanosterol	$C_{30}H_{52}O$	VI, R = A	150	+61	120	+60
Lanosterol	$C_{30}H_{50}O$	VI, R = B	140	+58	130	+57
Dihydrolanosterol	$C_{30}H_{50}O$	VII, R = A	159	+67	168	+86
Agnosterol	$C_{30}H_{48}O$	VII, R = B	169	+69	187	+92

7. *Group VII. Dimethyl- and Methylsterols*

The biochemical conversion of a trimethylsterol such as lanosterol (IX) to cholesterol (XIII) involves the loss of the methyl groups at 4,4 and 14. This elimination proceeds in all probability stepwise over dimethyl- and monomethylsterols. As a rule such sterols appear to have only a transitory existence. Nevertheless their presence in small but demonstrable quantities in sterol mixtures is to be expected. In an ingenious series of experimentations, Gautschi and Bloch (*55*) have recently proved the occurrence of a dimethylsterol, X, in rats killed soon after an injection of labeled acetate. This sterol and heretofore been an important "missing link" in the sequence leading from lanosterol (IX) to zymosterol (XI).

Monomethylsterols have also been found in sterol mixtures. 4α-Methyl-Δ^7-cholestenol (XII) was first obtained from rat feces (*56*). It has recently been shown to be identical with a cactus sterol, lophenol (*57*), $C_{28}H_{48}O$, m.p. 149–151°, $[\alpha]_D$ +5°; acetate, m.p. 121°, $[\alpha]_D$ +28°. It is now to be expected that all sterols of the groups (I) to (V) with the side chains A–M will also be found in nature as their mono-, di-, and trimethyl derivatives. As yet only a few additional representatives of this very large group of possible compounds have been found in plant sterol mixtures. The best characterized of these is citrostadienol or 4α-methyl-24-ethylidene-Δ^7-cholestenol (*58*) (ring structure of XII, and side chain I), $C_{30}H_{50}O$, m.p. 162–164°, $[\alpha]_D$ +24°; acetate, m.p. 142–143°, $[\alpha]_D$ +43°. It is obtainable from the oil of orange and grapefruit peel. The suggestion has been made that the long-known α_1-sitosterol, m.p. 164–166°, $[\alpha]_D$ −1.7°; acetate, m.p. 137°, $[\alpha]_D$ +29°; differs from citrostadienol only in the configuration around the 24,28-double bond (*59*).

III. Sterols of Bacteria and Protozoa

Dorée envisaged the possibility that sterols are not of primary importance to all forms of life and that animals might be found "into the composition of whose protoplasms it did not enter." Since then no animal entirely devoid of sterols has been found, but there is good evidence that sterols may not be necessary to all forms of life. It was first reported in 1889 (*60*) that no sterols could be detected in the lipids of tubercle bacilli by means of the conventional color reactions. Since then similar observations were made with diphtheria bacilli, "Bacillus Calmette-Guerin" (*Mycobacterium tuberculosis*), *Escherichia coli*, and *Mycobacterium lacticola*. They were verified by the particularly careful studies of von Behring (*61*) and of Anderson and his associates

(62). Von Behring interpreted his negative findings as demonstrating "that sterols are not fundamentally important compounds but make their appearance only in the course of phylogenetic development," and also "that sterols are connected with functions which are acquired later."

Equally unsuccessful have been attempts to detect sterols in certain primitive algae of the class of Myxophyceae (63). Such observations are of considerable interest for the Myxophyceae and bacteria are organisms without a well-formed nucleus. Such simultaneous absence of sterols and a well-developed nucleus suggests that sterols first appear in the course of evolution with the formation of the cell nucleus. Attractive as it is, this hypothesis is in need of broader experimental support. There are for example some indications that certain bacteria such as *Azotobacter chroöcoccum* (64) and *Escherichia coli* (65) contain small but definite amounts of sterols. These organisms should be reinvestigated under the most rigid exclusion of extraneous sterols. It should also be borne in mind that the absence of sterols in bacterial lipids has been shown only by negative color reactions and the lack of formation of insoluble digitonides. Saturated sterols, however, would not be detectable by conventional color reactions, and only slight structural modification of the sterol molecule, such as an inversion of the 3-hydroxyl group, leads to compounds not precipitated by digitonin. Until more reliable data have been gathered in this most attractive twilight zone of sterol production, we can state with certainty only that a number of bacteria and algae are devoid of the type of sterols normally met with in plants and animals.

Our present knowledge of the sterols of Protozoa, summarized in Table II, is quite fragmentary. Such paucity of information is mainly due to the extraordinary difficulties encountered in securing uniform material in quantities large enough for careful chemical studies. The few protozoans, whose sterols have been isolated, are relatively rich in lipids. These in turn contain the high percentage of unsaponifiable material which is so characteristic of fats of lower invertebrates (8). Cholesterol has been isolated from the fish parasite, *Eimeria gadi* (66) and the mycetozoan, *Labyrinthula minuta* (67). In the former it may well be of exogenous origin, but in the latter which has been grown on a sterol-free medium, its endogenous origin appears certain. Cholesterol is probably also the principal sterol of the malaria organism, *Plasmodium knowlesi* (68). Unidentified sterols have been isolated from the familiar, pelagic flagellate, *Noctiluca miliaris* (69), and the ciliate, *Tetrahymena geleii* (70).

It has been shown with certainty that some, but not all, protozoans

TABLE II

STEROLS AND STEROL REQUIREMENTS OF PROTOZOA

Classification	Total lipid (%)	Unsaponifiable fraction of lipid (%)	Sterol of unsaponifiable fraction (%)	Sterols Promoting growth
Class Mastigophora				
Order Dinoflagellidae				
Noctiluca miliaris (69)	12[a]	34	24, Unknown	—
Order Euglenoidida				
Paranema trichophorum (76)	—	—	—	Cholesterol, β-sitosterol, stigmasterol, poriferasterol, brassicasterol, ergosterol
Order Polymastigina				
Trichomonas columbae (77)	—			Cholesterol, 24-methylsterol, and ethylsterols
Trichomonas foetus (77)	—	—	—	Cholesterol
Trichomastix colubrorum (77)	—	—	—	Cholesterol
Trichomastyx batrachorum (77)	—	—	—	Cholesterol
Class Sarcoda				
Order Mycetozoa				
Labyrinthula vitellina (71) var. *pacifica*	—	—	—	Cholesterol, fucosterol, β-sitosterol
Labyrinthula minuta (67) var. *atlantica*			Cholesterol	None required
Order Amoebaea				
Entamoeba histolytica (72)	—	—	—	Cholesterol
Entamoeba terrapina (72)	—	—	—	Cholesterol
Entamoeba invadens (73)	—	—	—	None required[b]
Class Sporozoa				
Order Coccidiomorpha				
Eimeria gadi (66)	22[a]	36	81, Cholesterol	—
Plasmodium knowlesi (68)	29[a]	25	Cholesterol	—
Class Ciliata				
Order Holotrichidae				
Paramecium multicronucleatum (78)	—	—	—	Stigmasterol

TABLE II (*Continued*)

Classification	Total lipid (%)	Unsaponifiable fraction of lipid (%)	Sterol of unsaponifiable fraction (%)	Sterols Promoting growth
Paramecium aurelia (*74*)	—	—	—	Brassicasterol, fucosterol, β-sitosterol, but not cholesterol
Tetrahymena geleii (*70, 79–81*)	17[a]	25	40, m.p. 312°[c]	None required
Order Hypotrichidae				
Stylonychia pustulata (*75*)	—	—	—	Stigmasterol, β-sitosterol, but not cholesterol

[a] Based on dry weight.

[b] Sterols inhibit growth.

[c] An unlikely melting point for a sterol.

require dietary sterols for their normal growth. The sterol requirements differ, even among species of the same genus. Thus sterols are required for the growth of *Labyrinthula vitellina* (*71*) but not for *Labyrinthula minuta*, a cholesterol-producing organism. Although two amebas, species of the genus *Entamoeba*, require cholesterol (*72*), the growth of a third is inhibited by the addition of sterols to its culture medium (*73*).

The response of protozoans to sterols may be quite specific and related to the structure of the sterol side chain. Since the side chains often reflect the origin of the sterols, such specific responses may well be connected with the feeding habits of the organisms. Thus the ciliates *Paramecium aurelia* (*74*) and *Stylonychia pustulata* (*75*) do not respond to cholesterol, but only to Δ^5-sterols with methyl or ethyl substituents at C-24, which are the sterols most widely produced by plant life. In contrast, organisms such as *Paranema trichphorum* (*76*) and *Trichomonas columbae* (*77*) are not as discriminating and respond to a wide variety of sterols from the C_{27}-, C_{28}- and C_{29}-series.

IV. Sterols of Sponges (Porifera)

The first animal sterol clearly shown to be different from cholesterol was isolated fifty years ago from a Mediterranean sponge (*4*). It was given the appropriate name spongosterol. A few years later Dorée (*6*) obtained another sterol from the cosmopolitan sponge, *Cliona celata*, which he named clionasterol to indicate its difference from both cholesterol and spongosterol. When twenty-five years later the writer began his studies on invertebrate sterols, the first sponge under investigation, the common red New England sponge, *Microciona prolifera*, afforded yet another sterol, microcionasterol (*82*). A fourth sponge yielded a fourth new sterol, chalinasterol (*83*). The extraordinary fact that four different sponges had given four different sterols pointed to an almost unbelievable diversity of sterols among sponges. Indeed it seemed at this stage of the investigations that each family if not genus of sponges might produce its own characteristic sterol.

These astonishing results of such preliminary studies, fortuitous as they later proved to be, encouraged a comprehensive survey of sponge sterols. It was anticipated that such a survey would lead to a better understanding of the natural variations of the sterol molecule. It was hoped that in addition it might provide data which could be incorporated into the taxonomy of sponges, which is so bewildering to the uninitiated and understood by so few. An introduction of chemical criteria into the taxonomy of sponges would be traditional rather than novel. Even the earliest division of the Porifera into Keratosa, Calcarea, and Silicea was based on the chemical nature of their building material.

At present the sterol mixtures of more than fifty species of sponges have been isolated by the writer and his associates (*8*) (see Table III). Individual, identifiable sterols have been obtained from about one-half of them. Sufficient data are known about the remainder to assign their principal sterols to one or another of the classes previously discussed. With the beginning of a more systematic study of sponge sterols it soon became apparent that their diversity was not as spectacular as first indicated. Reinvestigation of the earlier sponge sterols proved them to be mixtures. Dorée's clionasterol was found to be composed of a monounsaturated sterol (II, R = L) for which the original name was retained, and a di-unsaturated compound, poriferasterol (II, R = M) (*84*). These two sterols were subsequently found to be the most common of the sponge sterols (*8*). The spongosterol of Henze was separated into cholestanol (IV, R = A) and the unique neospongosterol (IV, R = H) (*50*). Microcionasterol proved to be a rather complex mixture in which cholesterol (II, R = A) and cholestanol (IV, R = A) are the principal components (*85*). These observations were then of particular interest because they showed for the first time the presence of cholesterol in the primitive phylum of sponges and also the natural occurrence of cholestanol as a major rather than a minor sterol. The structure of chalinasterol, at first misunderstood, has since been shown to be that of 24-methylenecholesterol (II, R = D) (*38*).

Several other, new sterols have been obtained from sponges. Among these, chondrillasterol (III, R = M) is the only Δ^7-sterol known to occur in sponges (*86*). Aptostanol, m.p. 135°, $[\alpha]_D$ +22°; acetate, m.p. 134°, $[\alpha]_D$ +15°, is an unusual saturated sterol which occurs as the principal component in the sterol mixtures from several sponges of the family of Suberitidae (*87*). It was originally believed to be an isomer of ergostanol (IV, R = E), but it now appears more probable that it is an isomer of cholestanol (IV, R = A). Another sponge sterol of unknown structure is haliclonasterol (*88*), m.p. 141°, $[\alpha]_D$ −42°; acetate, m.p. 141°, $[\alpha]_D$ −46°. It gives an unusually characteristic acetate dibromide. The acetates of nearly all the unresolved sponge sterol mixtures show optical rotations sufficiently negative to indicate the predominance of Δ^5-unsaturated sterols (II).

As yet no sponges devoid of sterols have been found. In some keratinous sponges, however, the sterol content of the unsaponifiable fraction and of the total sponge is below the average for all sponges. These sponges, which are all members of the family of Spongiidae, when freshly caught discharge profusely a viscous liquid called gurry by the sponge fishermen. It contains about one-fourth of the total organic matter of the sponge and roughly one-half of its fat (*8*). This liquid, and the low sterol content, set these sponges apart from all others, including those of the family Verongiinae. The latter contain Δ^5-sterol mixtures in considerable amounts. It appears reasonable therefore to regard the Verongiinae as a separate family rather than a subfamily of Spongiidae. In its sterol content, and particularly the presence of cholesterol, the genus *Verongia* seems closely related to genus *Ianthella* of the family Dysiidae.

Studies on seven species of the genus *Haliclona* (order Haposclerina) have revealed a surprising lack of uniformity of sterols. While the sterol mixtures from *H. oculata* contain mainly 24-methylenecholesterol (chalinasterol) (II, R = D), the principal sterol of *H. variabilis* is poriferasterol (II, R = M), and the same sterol and clionasterol (II, R = L) are the major sterols of *H. permollis* and *H. coerulescens*. The sterol mixtures from *H. rubens* and *H. viridis* contain cholesterol in excess of 50%, and *H. longleyi* in contrast is the only known sponge to produce the unusual haliclonasterol (*88*).

At present approximately one hundred species have been assigned to the genus *Haliclona*. It appears to be a catchall, a polyphyletic aggregation of species and in need of a long overdue reappraisal. Any future revision of this genus might well take into account differences in sterol content, which suggest division into cholesterol, poriferasterol and clionasterol, 24-methylenecholesterol, and haliclonastrol bearing species.

Illustrative of the importance of sterol analysis to the taxonomy and phylogeny of sponges are the data derived from species of the orders Hadromerina and Halichondrina. Among the Hadromerina three species of the Choanitidae and two of Clionidae have afforded levorotatory mixtures of poriferasterol and clionasterol in nearly equal proportions (87). In significant contrast, nine species of Suberitidae were found to contain dextrorotatory, saturated sterols as the major components of their sterol mixtures. The sponges represented such different localities and habitats as the coast of New England (*Suberites ficus*), the Mediterranean Sea (*S. domuncula*), Bermuda (*Terpios fugax*) and (*Aaptos bergmanni*), Alaska (*S. suberea*), Hawaii (*T. zeteki*), and the great depths of the Atlantic Ocean (*Radiella sol, Weberella bursa* and *Polymastia infrapilosa*). Cholestanol is the principal sterol of some of the sponges, but in others its place is taken by aptostanol of as yet unknown structure. The predominance of saturated sterols in Suberitidae suggests a sterol metabolism significantly different from that of sponges belonging to other families of the order Hadromerina (87).

The usefulness of sterol analysis or of only the optical rotation of the crude sterol in the classification of sponges is best illustrated by the following example. The rather abundant Florida sponge, originally named *Suberites distortus* or *S. tuberculosus*, was found not to contain the saturated sterol with the positive rotation so typical of that family. Instead it afforded the familiar mixture of the levorotatory poriferasterol and clionasterol so typical of other families of this order. This purely chemical evidence led to the conclusion that the sponge had been misnamed and assigned to the wrong family. Subsequent taxonomic studies proved this conclusion to be correct and led to a reassignment of the sponge to the family of Choanitidae under the name of *Anthosigmella varians* (87).

In their sterol content, the Suberitidae show a much closer resemblance to two families of the order Halichondrina than to other families of its own order Hadromerina. Three species of Hymeniacidonidae representing such different regions as the coast of North Carolina (*Hymeniacidon heliophila*), the English Channel (*H. sanguinensis*), and Tasmania (*H. perlevis*) have all been found to contain cholestanol (IV, R = A) as their principal sterol. The same is almost true for the Halichondriidae. An unidentified species from the Attu Islands, *Halichondria melanodocea* from Florida, and the Japanese *H. panicea* (?) (*Reniera japonica*) all contain cholestanol as their major sterol. The common sponge of the coast of New England, *H. bowerbanki* originally misnamed *H. panicea*, however, contains about 80% of cholesterol in its

TABLE III
STEROLS OF SPONGES

Classification	Unsaponifiable fraction of fat (%)	Sterol of unsaponifiable fraction (%)	Sterols
Class Demospongia			
Order Keratosa			
Family Spongiidae			
Spongia obliqua (*89*)	34	4	Acetate, m.p. 120°; $[\alpha]_D - 33°$
Hircinia variabilis (*89*)	20	10	Δ^5-Sterols
Hippiospongia lachne (*89*)	40	6	Δ^5-Sterols
Oligoceras hemorrhages (*89*)	35	3	Δ^5-Sterols
Family Verongiinae			
Verongia fistularis (*89*)	25	71	Poriferasterol, clionasterol, cholesterol, and others
Verongia fulva (*89*)	24	44	Acetate, m.p. 116°; $[\alpha]_D - 40°$
Family Dysiidae			
Ianthella ardis (*89*)	23	73	Cholesterol, clionasterol, poriferasterol
Ianthella ianthella (*89*)	42	72	Cholesterol, clionasterol, poriferasterol
Order Haposclerina			
Family Spongillidae			
Spongilla lacustris (*6*, *90*)	—	—	Poriferasterol, clionasterol
Family Haliclonidae			
Haliclona oculata (*38*, *83*)	35	38	24-Methylenecholesterol
Haliclona longleyi (*88*)	38	74	Haliclonasterol
Haliclona rubens (*88*)	76	15	Cholesterol and others
Haliclona viridis (*88*)	30	68	Cholesterol and others
Haliclona variabilis (*88*)	32	63	Poriferasterol mainly
Haliclona permollis (*89*)	30	44	Poriferasterol, clionasterol
Haliclona coerulescens (*88*)	48	67	Poriferasterol, clionasterol
Family Desmacidonidae			
Isodictya deichmannae (*89*)	32	34	Acetate, m.p. 131°; $[\alpha]_D -43°$
Family Callyspongiidae			
Callyspongia vaginalis (*89*)	19	41	Poriferasterol, clionasterol

TABLE III (*Continued*)

Classification	Unsaponifiable fraction of fat (%)	Sterol of unsaponifiable fraction (%)	Sterols
Order Poecilosclerina			
Family Adociidae			
Adocia neens (*89*)	33	69	Acetate, m.p. 135°; $[\alpha]_D$ −43°
Family Myxillidae			
Tedania ignis (*89*)	24	66	Poriferasterol, clionasterol
Lissodendoryx isodictyalis (*89*)	36	50	Acetate, m.p. 135°; $[\alpha]_D$ −50°
Family Microcionidae			
Microciona prolifera (*85*)	53	48	Cholesterol, cholestanol
Order Halichondrina			
Family Axinellidae			
Homaxinella rudis (*89*)	42	60	Poriferasterol, clionasterol
Pseudaxinella rosacea (*89*)	37	51	Poriferasterol, clionasterol
Axinella polycapella (*89*)	37	59	Acetate, m.p. 141°; $[\alpha]_D$ −40°
Family Halichondriidae			
Halichondria bowerbanki (*85*)[a]	37	57	Cholesterol (80%)
Halichondria sp. (Attu) (*89*)	28	56	Cholestanol and others
Halichondria melanodocea (*89*)	38	65	Cholestanol (60–70%)
Halichondria sp. (Japan) (*89*)	45	58	Cholestanol
Family Hymeniacidonidae			
Hymeniacidon heliophila (*85*)	38	68	Cholestanol
Hymeniacidon sanguinensis (*89*)	49	35	Cholestanol
Hymeniacidon perlevis (*89*)	44	75	Cholestanol
Order Hadromerina			
Family Suberitidae			
Suberites ficus (*50*)[b]	36	70	Cholestanol, neospongosterol
Suberites domuncula (*4, 50*)	—	—	Cholestanol, neospongosterol
Suberites suberea (*87*)	—	—	Dextrorotatory sterol
Terpios fugax (*87*)	35	62	Cholestanol and others
Terpios zeteki (*87*)	41	64	Cholestanol and others
Weberella bursa (*87*)	64	55	Cholestanol, aptostanol
Polymastia infrapilosa (*87*)	38	66	Cholestanol, aptostanol

TABLE III (*Continued*)

Classification	Unsaponifiable fraction of fat (%)	Sterol of unsaponifiable fraction (%)	Sterols
Radiella sol (87)	37	55	Aptostanol
Aaptos bergmanni (87)	33	69	Aptostanol
Family Clionidae			
Cliona celata (84)			Poriferasterol, clionasterol
Cliona carriboea (87)	27	58	Poriferasterol, clionasterol
Family Choanitidae			
Spheciospongia vesparia (84)	31	70	Poriferasterol, clionasterol
Spheciospongia othello (87)	46	50	Poriferasterol, clionasterol
Anthosigmella varians (87)	48	58	Poriferasterol, clionasterol
Order Epipolasida			
Family Tethyidae			
Tethya actinia (89)	30	90	Clionasterol, and others
Cryptotethia crypta (91)	56	70	Poriferasterol, clionasterol
Order Choristidae			
Family Tetillidae			
Cynachyra cavernosa (89)	46	73	Clionasterol and others
Family Geodiidae			
Geodia gibberosa (89)	32	60	Acetate, m.p. 136°; $[\alpha]_D$ −46°
Family Craniellidae			
Craniella crania (88)[c]	34	80	24-Methylene cholesterol
Order Carnosa			
Family Chondrillidae			
Chondrilla nucula (86)	40	86	Chondrillasterol
Class Calcispongia			
Order Asconosa			
Leucetta floridana (89)	18	14	Acetate, m.p. 132°; $[\alpha]_D$ −46°

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[a] Formerly named *Halichondria panicea.*

[b] Formerly named *Suberites compacta.*

[c] Formerly named *Tetilla laminaris.*

sterol mixture. It may therefore be regarded as significantly different from other species of this genus.

The data on sterols from other sponges of the class of Demospongia are too fragmentary to permit their correlation to taxonomic aspects. It is of interest, however, that only *Chondrilla nucula* (*86*) of the order Carnosa has been found to contain a Δ^7-sterol, chondrillasterol (III, $R = M$), as a major component of the sterol mixture. This family includes sponges of such unspongelike appearance and consistency as to merit its separation into a special order. As yet nothing is known about the sterols of the very attractive glass sponges of the class of Hyalospongiae. Sponges belonging to the class of Calcispongiae are generally quite small and poor in organic matter. The one exception is the Bermuda species *Leucetta floridana*, which is of comparatively gigantic size. The sterol content of its unsaponifiable fraction appears to be quite low, and this may well be true for other sponges of this class (*89*).

V. Sterols of Coelenterates

The unusual diversity of sponge sterols, appealing as it is to the steroid chemist, should not be overvalued in tracing the biochemical history of the sterols. The sponges constitute a rather special group of animals. They are regarded as a very early and probably polyphyletic branch off the main stem of evolution, and hence as ancestrally unrelated to animals of other phyla. It is therefore of considerable interest that a similar variety of sterols is encountered also among the coelenterates, a phylum of animals less primitive than the sponges and more representative of the main stream of evolution (*92*).

The lipid content of coelenterates is generally rather high, particularly when expressed in terms of the organic matter of the animal rather than its total weight. In certain sea anemones the lipids constitute as much as one-third of the dry weight of the animal. The lipids are unusually rich, as high as 50%, in such waxlike materials as cetyl palmitate (*93*) or myristyl myristate (*92*). These esters or their homologs may be regarded as some of the most typical components of coelenterates (*8*). Because of their presence, the unsaponifiable fractions of coelenterate lipids are generally so rich in higher aliphatic alcohols as to make the separation of the sterols at times rather difficult.

Our knowledge of coelenterate sterols is still quite fragmentary. It includes but one representative each of the classes Hydrozoa and Scyphozoa (see Table IV). They both contain cholesterol, which is also present in many of the sea anemones. Because they are quite conspicuous, easy to collect, and rich in lipids, the sea anemones have so far been the preferred source of material for coelenterate sterols. In a few species cholesterol has been found replaced as major sterol by 24-methylenecholesterol (*21*) or unidentified sterols or sterol mixtures.

TABLE IV
STEROLS OF COELENTERATES

Classification	Unsaponifiable fraction of lipids (%)	Sterol of unsaponifiable fraction (%)	Sterols
Class Hydrozoa			
Order Siphonophora			
Velella spirans (97)	5.3	—	Cholesterol
Class Scyphozoa			
Order Rhizostomae			
Rhizostoma cuvieri (98)	3	—	Acetate, m.p. 115–116°
Class Anthozoa			
Order Gorgonaceae			
Plexaura flexuosa (96)	36	31	"Gorgosterol," m.p. 185°
Xiphogorgia sp. (*96*)	—	—	Sterol, m.p. 140°;
Order Zoanthidea			
Zoanthus proteus (38)	39	30	24-Methylene cholesterol
Palythoa mammilosa (21)	25	47	Palysterol
Order Actiniaria (sea anemones)			
Bolocera tuediae (92)	23	14	Cholesterol
Actinostola callosa (92)	17	—	Cholesterol
Anthopleura japonica et al. (95)	34	45	β-Sitosterol
Anthopleura elegantissima (89),	—	—	Cholesterol
Actinia equina (6)	—	—	Cholesterol
Tealia crassicornis (6)	—	—	Cholesterol
Calliactis japonica (99)	32	55	Cholesterol
Chondylactis gigantea (21)	24	10	24-Methylenecholesterol
Anemonia sulcatis (94)	—	—	"Actiniasterol"
Metridium marginatum (89)	18	45	Cholesterol
Order Madreporaria (corals)			
Meandra areolata (96)	—	14	Cholesterol and others
Porites porites (89)	—	—	Brassicasterol and others
Oculina diffusa (96)	24	9	Acetate, m.p. 136°
Madrepora cervicornis (96)	33	7	Sterol, m.p. 176.5°

Actiniasterol, once believed to be a uniform, new sterol has since been shown to be a mixture of two Δ^5-sterols of unknown identity (*94*). Although cholesterol has been identified as the major sterol of the sea anemone *Anthopleura elegantissima*, from the west coast of the United States (*89*), a mixture of *Anthopleura* species from Japan have afforded a sterol believed to be β-sitosterol (*95*). These contradictory observations suggest either that the sterols of *Anthopleura* reflect the diet of the animals, or more likely that one or another of the species has been misidentified or placed within the wrong family.

A more interesting diversity of sterols is encountered among the colonial anthozoa belonging to the orders of gorgonias, Madreporaria (corals), and zoantharia, the colonial sea anemones. Two of the latter have each given a different sterol. The one is the rather widely distributed 24-methylenecholesterol, obtainable from the animal in a surprisingly uniform state (*38*, *92*). The other, provisionally named palysterol, appears to be a new compound of unknown structure, m.p. 140°, $[\alpha]_D$ −47°; acetate, m.p. 152°, $[\alpha]_D$ −52° (*21*). Sterols or sterol mixtures melting higher than the natural sterols now known have been isolated from gorgonias and corals. One of the former contains a sterol tentatively called gorgosterol (*96*), m.p. 184–185°, $[\alpha]_D$ −45°; acetate, m.p. 140° clear at 152°, $[\alpha]_D$ −56°; and one of the corals, a sterol of m.p. 176–177° (*96*). Such exceptionally high melting points set these sterols apart from other levorotatory Δ^5-sterols and suggest the presence of a side chain or other structural features not heretofore encountered. A more systematic study of these sterols should furnish data significant to the understanding of the biochemical evolution of sterols. It is of interest to note that all major coelenterate sterols which have been observed so far belong to group II, the Δ^5-sterols. They are generally accompanied by small amounts of the provitamin D sterols of group I. No significant quantities of saturated sterols have yet been found, which are so characteristic of certain sponge families; nor any of the Δ^7-sterols of group III, typical of certain sponges, mollusks, and echinoderms.

VI. Sterols of Ctenophores to Annelids

With but very few exceptions nothing appears to be known about the sterols of the many and diverse animals which have been assigned to the six or more phyla generally placed between the Coelenterata and Arthropoda. Substantial quantities of cholesterol have been isolated from the parasitic flatworms (Plathelminthes) such as the fish tapeworm, *Botriocephalus latus* (*Diphyllobotrium latum*) (*100*), the liver fluke, *Fasciola hepatica* (*101*), and the cat and beef tapeworms, *Taenia taeniaeformis* (*102*) and *T. saginata* (*103*). Cholesterol is also the principal sterol of *Ascaris lumbricoides* (*104*), the eelworm of the phylum of roundworms (Nemathelminthes). The common earthworm, *Lumbricus terrestris*, of the phylum Annelida, has been shown to contain cholesterol as the principal sterol (*6*, *105*), accompanied by as much as 20% of ergosterol (I, R = F) and also some other unidentified sterols (*106*).

VII. Sterols of Arthropods

A. STEROLS OF CRUSTACEANS AND MYRIAPODS

This phylum, so rich in species, embraces the Crustacea, Arachnoidea, Myriapoda, and Insecta. With but very few exceptions nothing is known about the sterols of these animals. No systematic and comprehensive studies have been done on the comparative biochemistry of the sterols of Crustacea, a class so abundant in readily accessible species. Fragmentary observations point to a wide distribution of cholesterol among representatives of many orders. In the subclass of Entomostraca, cholesterol has been found in a brine shrimp of the primitive order of Branchiopoda (*107*). Cholesterol has also been obtained from the oil of Copepoda (*108*), from *Cipridina* of the order Ostracoda (*109*), and from the barnacle, *Balanus glandula*, of the order Cirripedia (*33*). In the subclass of Malacostraca, cholesterol appears to be the principal, but not only, sterol of *Ligia* (syn. *Ligyda*) *exotica* (*20*), the common small crustacean of the order Isopoda which lives above high-tide mark. Cholesterol is the principal sterol of many if not all representatives of the order Decapoda. Among the shrimps and prawns it has been isolated from *Palaemon nipponensis* (*110*) and from shrimp waste. The latter material might some day become a commercial source of cholesterol (*111*). The Norway lobster, *Nephrops norvegicus* (*112*), the freshwater crayfish, *Cambarus clarkii* (*110*), and such crabs as *Carcinus maenas*, the green crab (*6*), *Portunus plicatus* (*113*), *Eriphia spinifrons* (*113*), and *Cancer magister* (*114*), all contain cholesterol.

An unfortunate *a priori* assumption that cholesterol is the crustacean sterol par excellence is probably responsible for the paucity of data. What interesting and important discoveries may yet be made in this field is best illustrated by recent work on barnacle sterols. This mixture also consists largely, 60%, of cholesterol but contains in addition about 35% of 24-dehydrocholesterol (II, R = B) (*33*). This sterol is one of the interesting final links in the chain of biosyntheses leading to cholesterol. It had first been found in only very small quantities in the chick embryo, and it had been named desmosterol (*35*). A careful and near-quantitative study of the sterol mixtures of crustaceans may be expected to reveal additional interesting sterols and to provide data correlating the taxonomy and evolution of these animals to their sterol content.

Much less is known about the sterols of the Arachnoidea, the spiders, and the Myriapoda, the centipedes and millepedes. Cholesterol has been isolated from the "living fossils," the horseshoe crabs, *Limulus polyphemus* (*96*) and *L. longispina* (*Trachypleus tridentatus*) (*115*) of the Arachnoidea. The sterol is accompanied by some unidentified diunsaturated Δ^5-sterol (*115*).

The fatty extract of the red millepede, *Strongylosoma tambanum* (*116*), has found some medicinal use in Japan under the name "Akayusade" oil. It contains about 20% of unsaponifiable material from which yasudosterol, a poorly identified compound, has been isolated.

B. Sterols of Insects

About fifty years ago the sterols of four species of insects had already been isolated and investigated. The Spanish fly, *Lytta vesicatoria*, which is a phytophagous beetle, had been found to contain cholesterol (*7*). It had been well identified as its dibromide, isobutyrate, and benzoate. The common cockchafer of Europe, *Melolontha vulgaris* (*7*), and the cockroach, *Blatta orientalis* (*6*), had given sterol mixtures among which cholesterol was suspected to be one of the major components. The sterol from the chrysalis oil of the silk moth, *Bombyx mori*, became the subject of a controversy soon after its first isolation by Lewkowitsch in 1907 (*117*). While this author believed it to consist essentially of cholesterol, Tsujimoto held it to be sitosterol (*118*). About the same time two Italian investigators regarded it as a new and uniform sterol, which they named bombicesterol (*5*).

Since these early and preliminary investigations of half a century ago our knowledge of insect sterols has made only very little progress. Bombicesterol has been reinvestigated and declared a mixture of cholesterol and a sitosterol-like product (*119*). This conclusion has been challenged by Kawasaki (*120*), who has reaffirmed his belief in the existence of a uniform bombicesterol in continuation of the old controversy (*121*). Inagosterol, obtained from the oil of locusts of the probable species *Oxya velox* and *Oxya vicina* (*122*), has been shown either to be identical with bombicesterol or the mixture of cholesterol and the sitosterol-like compound. Russian and Mexican cantharides (Spanish flies) have been found to contain β-sitosterol (*123*) in contrast to cholesterol previously reported. Cholesterol has been isolated from "ants' eggs," the pupae of ants (*123, 124*), from wasps such as *Vespa mandarina* (*125*), and from the common cabbage butterfly, *Pieris brassica* (*126*). The last observation is of particular interest because the cholesterol was satisfactorily identified, and no other sterols were found in spite of a careful search. This investigation, which has been overlooked by most investigators, proves conclusively that cholesterol may indeed be the nearly exclusive sterol of a phytophagous insect. In general, however, cholesterol is accompanied by one or possibly more sterols which unfortunately have never been fully identified. Because of their physical properties and those of their acetates, they have been called sitosterol or sitosterol-like products. It should be remembered, however, that such similarities no longer imply identity with plant sterols of the order C_{29}. The companion sterol might also be 22-dehydrocholesterol, whose physical properties are very similar to those of β-sitosterol (see under sterols). A complete knowledge of the identity of the companion sterols will be of greatest importance to the understanding of the role played by the sterols in the nutrition of insects.

Contrasted with the extraordinary dearth of information on the sterols of so large and so important a class of animals, is the rapidly expanding knowledge of the sterol requirements of insects. At least twenty different species of insect larvae, representative of the orders Orthoptera, Coleoptera, Lepidoptera, and Diptera, have been found to require sterols as growth factors, and none have yet been found which do not. It appears at present that a dependence on exogenous sterol is characteristic of all insects. A full discussion of this interesting phenomenon, which has recently been adequately reviewed (*127*, *128*), would go beyond the scope of the present survey. Suffice it to say that the species which have so far been studied may roughly be divided into two groups on the basis of their natural food and their sterols requirements. The phytophagous species are less specific in their requirements, and respond to cholesterol and its C-24 homologs. *Dermes vulpinus*, the hide beetle, on the other hand, whose larva normally feeds on products of animal origin, is known to utilize only sterols with the carbon skeleton of cholesterol (*129*).

If the phytophagous caterpillar of the cabbage butterfly is dependent on the C_{28}- and C_{29}-sterols for its food, it must possess a mechanism for the elimination of the alkyl groups at C-24 to form cholesterol, which is the only sterol found in the adult insect. Such eliminations can indeed be shown to occur. The phytophagous flour beetle, *Tribolium confusum*, can utilize cholesterol, ergosterol, and sitosterol. It converts the C_{28}- and C_{29}-sterols into compounds tentatively identified by color reactions as cholesterol and 7-dehydrocholesterol (*130*). A more ingenious demonstration of the conversion of higher homologs to C_{27}-sterols has recently been given by E. D. Bergmann (*131*). The larvae of the fly, *Musca vicina*, were grown on a special bran diet containing a C_{29}-sterol, sitosterol. The pupae were then fed to larvae of the hide beetles, *Dermes vulpinus* and *D. cada-*

verinus, which grew on this diet. Since these beetles require cholesterol or 7-dehydrocholesterol it follows that the larvae of the flies had eliminated the supernumerary carbon atoms at C-24 of the plant sterols and had converted them into sterols capable of sustaining the growth of *Dermes* larvae.

VIII. Sterols of Mollusks

Sterol mixtures have been isolated from a comparatively large number of mollusks. Although they are in most instances rather complex and have resisted attempts to separate them into their components, they have nevertheless furnished adequate physical data to point toward an interesting relation between the sterols of mollusks and their taxonomy. The significance of these relations is exceeded only by those found for the Echinodermata. The data on mollusk sterols so far available have been summarized in Table VII.

The class of the Amphineura is of particular interest. These chitons and related forms had been placed among the worms, Annelida, prior to 1881 when Spengel placed them among the Mollusca. Since then they have been thought to occupy a key position in this phylum and to be but slightly differentiated from the prototype mollusk. It has been shown independently and convincingly both in the United States (*132*) and Japan (*133, 134*) that these primitive mollusks contain Δ^7-sterols (III), among which Δ^7-cholestenol (III, $R = A$) appears to be the principal component. This fact sets the Amphineura, or at least species from the subclass of Polyplacophora apart from all other classes of mollusks which have been found to contain Δ^5-sterols (see Table VII). Inclusion of data on the sterols of species from the subclass of Aplacophora, however, will be necessary before a biochemical evaluation of the peculiar position of the Amphineura in the phylum of Mollusca may be attempted. Most welcome will be data on the sterols of *Neopilina galathea.* This very important mollusk, which was only recently discovered, is a "living fossil," the only known survivor of the class of Monoplacophora.

It is also unfortunate that nothing is known about the sterols of mollusks from the class of Scaphopoda, which were once believed to occupy a position between the snails and the bivalves. They are held to be very primitive mollusks, and so aberrant in structure as to warrant a class for themselves.

All sterol mixtures which have been isolated from Gastropoda, the snails and slugs, are rather levorotatory, indicative of the predominant presence of Δ^5-sterols. An adequate number of species from the subclass Prosobranchia and its orders Archeogastropoda, Mesogastropoda, and Stenoglossa have been investigated to show convincingly that cholesterol is the major component of their sterol mixtures (see Table VII).

The situation is not as clear for species from the subclass of Ophisthobranchia. One species each from the orders Pleurocoela (Tectibranchia) and Acoela (Nudibranchia) have afforded unusual sterol mixtures with a single ultraviolet absorption peak at 277–278 mμ. From two species of the order Pteropoda, sterol mixtures have been obtained in which the major component is a substance tentatively called pteropodasterol, acetate, m.p. 136° (*135*), rather than cholesterol. This differentiation from other Gastropoda is of considerable interest. The pelagic, free-swimming snails belonging to the Pteropoda had long been considered by zoologists to belong to a class equivalent in value to the Gastropoda, and had even been regarded as the ancestors of the Cephalopoda. The unusual sterol sets these snails indeed apart, at least from other species of the subclass of Prosobranchia. It does not, however, point to a relationship to the Cephalopoda, which contain cholesterol as the nearly exclusive component of their sterol mixture.

The data available for sterols of species from the subclass of Pulmonata, the land and freshwater snails, are too inadequate to permit even preliminary generalizations. It appears, however, that they also contain cholesterol as the major sterol, accompanied, however, by rather large quantities of $\Delta^{5,7}$-sterols. Considerable quantities of such provitamins D have also been found in marine Gastropoda (see Table VII). The familiar whelk, *Buccinum undatum*, for example, has been shown to contain 78% of 7-dehydrocholesterol (I, R = A) in its sterol mixture (*106*).

The sterol mixtures of the Pelecypoda, the bivalves, are of particular interest. They also contain cholesterol (*136*), but in smaller quantities than the mixtures obtainable from many of the Gastropoda. The other sterols, complex a mixture as they may be, afford acetates that, even when crude, have a melting point significantly higher than cholesteryl acetate, m.p. 115–116°. A mollusk sterol acetate mixture melting between 110° and 120° points toward cholesteryl acetate as its major component and hence to Gastropoda as its source material. A melting point above 130°, however, indicates the predominant presence of other steryl acetates and Pelecypoda as the source material. It should be borne in mind, however, that these differences are subject to seasonal variations, and that exceptions have been noted as in the case of the Pteropoda. Nevertheless such figures are of help in the analysis of the data given in Table VII.

In 1934 Bergmann (*137*) and Tsujimoto and Koyanagi (*138*) independently made the observation, then unexpected, that cholesterol is not the only, or even the principal, sterol of bivalves. Bergmann recognized the sterol as a di-unsaturated compound which he named ostreasterol. He subsequently found it to be identical with chalinasterol for which structure (III, R = H) had erroneously been proposed (*17*). The Japanese investigators named their compound conchasterol. Other names such as pectosterol, meretristerol, shakosterol, magakisterol, and most recently pincsterol were proposed for similar or different sterols isolated from Japanese mollusks (*136*). In a reinvestigation of bivalve sterols, Idler and Fagerlund were the first to recognize the presence of 24-methylenecholesterol (II, R = D) and in doing so solved some of the more puzzling aspect of bivalve sterol mixtures (*37*). Subsequently the identity of ostreasterol (chalinasterol) with 24-methylenecholesterol was demonstrated (*38*).

In Table V the physical properties of synthetic 24-methylenecholesterol are compared with those of other bivalve sterols. It appears reasonable to assume that in addition to ostreasterol, pectosterol, pincsterol, conchasterol, and meretristerol consist essentially of 24-methylenecholesterol. The last two sterol mixtures with high-melting acetates probably contain significant quantities of brassicasterol (II, R = F). The presence of this sterol in mollusks has clearly been demonstrated (*136*). Brassicasterol also appears to be the principal component of the bivalve sterols shakosterol and magakisterol. It is of interest to note that two of the identified bivalve sterols are di-unsaturated isomers of the order $C_{28}H_{46}O$ and that the third is the mono-unsaturated choles-

TABLE V
DI-UNSATURATED STEROLS OF BIVALVES

Name	Sterol		Acetate		Acetate Br_4 (m.p., °C.)	Benzoate (m.p., °C.)
	m.p., °C.	$[\alpha]_D°$	m.p., °C.	$[\alpha]_D°$		
24-Methylenecholesterol (synthetic) (*38*, *39*)	145	−42	135	−47	156–158	151–152
24-Methylenecholesterol (natural) (*37*)	142	−35	136	−42	—	148–153
Ostreasterol (chalinasterol) (*17*)	147	−42	135	−46	154–156	151–152
Pectosterol (*140*)	134–137	−35	137	—	—	—
Pincsterol (*141*)	137	−45	134	−44	—	141
Conchasterol (*138*)	133–134	—	145	—	—	—
Meretristerol (*142*)	135	−44	145	−50	—	—
Brassicasterol	148	−60	152	−62	209–216	163
Shakosterol (*143*)	147	—	157	—	—	—
Magakisterol (*144*)	153	−47	150	−52	—	—

terol. The search for other components is complicated by the fact, observed both in the United States and in Japan (*139*), that sterol mixtures undergo fluctuations in composition, the significance of which is not yet understood.

Mollusk sterols and in particular certain bivalve sterols are among the richest natural sources of $\Delta^{5,7}$-sterols (I), or provitamins D. This interesting fact was discovered in connection with a systematic search for natural precursors of vitamin D which upon irradiation would afford a product of the antirachitic activity of cod liver oil rather than of irradiated ergosterol. The characteristic ultraviolet spectrum of the $\Delta^{5,7}$-sterols greatly facilitates their detection and quantitative determination. The sterols from certain mussels, such as *Modiolus demissus*, were found to be sufficiently rich in provitamins D to warrant their commercial exploitation for the manufacture of vitamin D preparations (*145*).

The isolation of a pure, identifiable $\Delta^{5,7}$-sterol from bivalves has been a rather vexing problem. The general complexity of the bivalve sterols appears to be reflected in the number of $\Delta^{5,7}$-sterols present in the sterol mixtures. Mixtures of $\Delta^{5,7}$-sterols are particularly difficult to separate chromatographically. Even systematic studies on commercial mussel sterols, once available in very large quantities, have not led to the isolation of a structurally identifiable $\Delta^{5,7}$-sterol. It could only be suggested on the basis of rather tenuous qualitative and quantitative evidence that the mussel provitamins consist of about equal parts of 7-dehydrocholesterol (I, R = A) and a mixture containing ergosterol (I, R = F), the unknown $\Delta^{5,7,22}$-cholesta-trienol (II, R = C), and provitamin D_x (*146*). In contrast, other investigators obtained none of these sterols from the mussel *Modiolus demissus*, but instead a compound of the order C_{29}, provisionally called provitamin D_m (*147*).

In Table VI the known $\Delta^{5,7}$-sterols are compared with those isolated from mollusks. As mentioned in the introductory chapter, the data for 7-dehydrositosterol and 7-dehydroclionasterol must be regarded as provisional. Similarities of certain data suggest the identity of certain mollusk sterols and known 7-dehydrosterols. Thus, the identity of the *Littorina* provitamin D with 7-dehydroclionasterol has been suspected (*148*). Corbisterol, which Matsumoto and Toyama have isolated from Japanese bivalves, appears to be a uniform compound. It is a triunsaturated compound and it is believed to be identical with 7-dehydrostigmasterol (I, R = K) (*149–152*). This formula, however is not in accord with the antirachitic activity of irradiated corbisterol. In the rat test its activity is the same as that of irradiated ergosterol (I, R = F) and 7-dehydrocholesterol (I, R = A), but significantly higher than that of the irradiation product of 7-dehydrostigmasterol (I, R = K) suspected of being identical with corbisterol (*153*). From a comparison of the sizes of the side chains and vitamin D activities the conclusion has been drawn that corbisterol is of the order C_{28} rather than C_{29} (*153*).

TABLE VI
PROVITAMINS D OF MOLLUSKS

	Sterol		Acetate	
	m.p., °C.	$[\alpha]_D^\circ$	m.p., °C.	$[\alpha]_D^\circ$
7-Dehydrocholesterol	150	−114	130	−85
22-Dihydroergosterol	153	−109	158	−75
Ergosterol	168	−130	181	−90
7-Dehydrocampesterol	165	−109	—	—
7-Dehydro-β-sitosterol	145	−116	152	−71
7-Dehydroclionasterol	138	−98	140	−72
7-Dehydrostigmasterol	154	−104	172	—
Corbisterol (*149–152*)	154	−114	173	−80
Littorina sterol (*147*)	137	−124(C_6H_6)	136	−85(C_6H_6)
Provitamin D_m (*147*)	127	−108	138	−72
Mytilus sterol (*147*)	150	−118	161	−85
Pinna pectinata sterol (*154*)	123	−105	122	−75
Tonna luteostoma sterol (*155*)	119	−110	116	−74

The Japanese investigators have made a number of additional interesting, if as yet rather preliminary, observations. They have isolated $\Delta^{5,7}$-sterols of unusually low melting points. The properties of sterols from *Pinna* and *Tonna* (*148*) (Table VI) are reminiscent of those of provitamin D_m. The *Tonna* sterol is thought to be tetraunsaturated with two double bonds in the side chain and probably identical with the unknown $\Delta^{5,7,22,25}$-cholestatetraenol (I, R = P) (*155*). Another sterol, doubly unsaturated in the side chain, the 24(28)-dehydroergosterol (I, R = O) had previously been isolated from yeast sterols. It is also a rather low-melting compound (m.p. 118–120°) (*19*).

The Japanese investigators have also observed a curious dichotomy of behavior among the $\Delta^{5,7}$-sterols of mollusks (*149*). A considerable fraction of these sterols is not precipitated by digitonin. Since the loss of precipitation by digitonin with retainment of the characteristic ultraviolet absorption is one of the first steps of the *in vitro* conversion of a provitamin D to the vitamin, these unknown $\Delta^{5,7}$-sterols may well represent one of the steps in the natural formation of vitamin D.

Cholesterol appears to be the principal sterol of the Cephalopoda, the most advanced class of the Mollusca. It has been isolated from *Sepia*, from squids and octopus, and more recently also from commercial Japanese cuttlefish oil (*156*) (see Table VII).

TABLE VII
STEROLS OF MOLLUSKS

Classification	Unsaponifiable fraction of fat (%)	Sterol of unsaponifiable fraction (%)	Sterol or m.p. of steryl acetate	$\Delta^{5,7}$-Sterols in mixture (%)
Class Amphineura				
Subclass Polyplacophora (chitons)				
Family Acanthochitidae				
Acanthochiton rubrolineatus (134)	23	44	Δ^7-Sterol	—
Family Chitonidae				
Chiton tuberculatus	11	30	Δ^7-Cholestenol	—
Liolophura japonica (133, 134)	22	53	Δ^7-Cholestenol	—
Class Gastropoda				
Subclass Prosobranchia				
Order Archeogastropoda				
Family Haliotidae (ear shells)				
Haliotis gigantea (157) (abalone)	23	43	Cholesterol	0.3
Family Patellidae				
Cellana nigrolineata (138)	24	—	114–115°	—
Cellana toruema (158)	24	—	114°	—
Family Trochidae				
Tegula argyrostoma (159)	16	59	Cholesterol	1.2
Tegula xanthostigma (160)	11	—	Cholesterol	—
Monodonta labio (158)	25	72	Cholesterol	0.2
Family Turbinidae (turban shells)				
Lunella coronata coreensis (161)	23	69	Cholesterol	0.6
Turbo cornutus (161)	30	63	Cholesterol	0.5
Family Neritidae				
Nerita peleronta (bleeding tooth shell) *(162)*	—	—	Cholesterol	—
Order Mesogastropoda				
Family Viviparidae				
Viviparus japonicus (163)	26	47	Cholesterol[a]	6.0
Viviparus malleatus (163)	23	50	Cholesterol[a]	6.0
Family Littorinidae (periwinkles)				
Littorina littorea (148)	—	—	Cholesterol	9.6
Order Stenoglossa				
Family Muricidae				

TABLE VII (*Continued*)

Classification	Unsaponifiable fraction of fat (%)	Sterol of unsaponifiable fraction (%)	Sterol or m.p. of steryl acetate	$\Delta^{5,7}$-Sterols in mixture (%)
Urosalpinx cinereus (oyster drill) *(164)*	—	—	Cholesterol	—
Rapana thomasiana (138)	15	—	Cholesterol	—
Family Nassidae				
Nassa obsoleta (164)	—	—	Cholesterol	—
Family Buccinidae				
Buccinum perryi (164)	27	67	Cholesterol	10.0
Hemifusus ternatus (161)	25	36	Cholesterol[a]	—
Busycon (Fulgur) canaliculatum (whelk) (139)	—	—	Cholesterol	—
Subclass Opisthobranchia				
Order Pleurocoela				
Family Philinidae				
Philine japonica (99)	—	29	Unusual[b]	—
Order Pteropoda (sea butterflies)				
Family Spiratellidae				
Limacina helicina (135)	11	62	Pteropodasterol, m.p. 136°	—
Family Clionidae				
Clione limacina (135)	14	14	Pteropodasterol	—
Order: Acoela (sea slugs)				
Family Dorididae				
Actinocyclus japonicus (99)	—	34	Unusual[b]	—
Subclass Pulmonata				
Order Stylommatophora				
Helix pomatia (vineyard snail) *(106)*	—	—	Cholesterol	9.6
Euhadra herklotsi (165)	27	71	Cholesterol	5.0
Arion empiricorum (land slug) *(106)*	—	—	Cholesterol	22
Incillaria confusa (166)	—	—	Sitosterol (?)	0.3
Class Pelecypoda				
Order Taxodonta				
Family Arcidae				
Anadara subcrenata (161)	25	63	140	1.9
Order Anisomyaria				
Family Mytilidae				
Mytilus edulis (161)	10	51	115–130[c]	1.2
Mytilus planatus (167)	—	—	—	—
Volsella modiolus (136)	16	—	130+	—

TABLE VII (*Continued*)

Classification	Unsaponifiable fraction of fat (%)	Sterol of unsaponifiable fraction (%)	Sterol or m.p. of steryl acetate	$\Delta^{5,7}$-Sterols in mixture (%)
Modiolus demissus (*136, 168*)	—	—	152[d]	—
Family Pteriidae				
Pinna pectinata japonica (Spanish oyster) (*154*)	20	65	120–140	13.2
Pinctada martensis (pearl oyster) (*141*)	—	—	133–134[e]	—
Family Ostreidae (oysters)				
Ostrea gigas (*37, 138*)	13	—	136–137	—
Ostrea virginica (*137*)	—	—	135–136[g]	—
Family Pectinidae (scallops)				
Pecten caurinus (*168*)	—	—	136–137[h]	—
Pecten yessoensis (*140*)	—	—	137	—
Chlamis farreri nipponensis (*158*)	29	71	133–135	0.73
Order Eulamellibranchia				
Family Unionidae				
Cristaria spatiosa (*158, 163*)	32	39	120–130	7–12
Anadonta woodiana lauta (*149*)	22	49	130–147	11–14
Family Cardiidae				
Cardium cordis (*168*)	—	—	136–137[h]	—
Family Tridacnidae				
Tridacna gigas (giant clam) (*160*)	—	—	156–157[j]	—
Family Veneridae				
Meretrix meretrix (*142*)	13	—	137–138[k]	—
Dosinia japonica (*161*)	45	46	135–136	0.22
Saxidomus giganteus (*168*)	—	—	136–137[h]	—
Venerupis semidecussata (*169*)	—	—	120–140[l]	10
Tapes philippinarum (*157*)	15	—	130–137	—
Family Macridae (surf clams)				
Spisula sachalinensis (*158*)	—	—	132	—
Family Cyrenidae				
Corbicula leana (*158*)[m]	—		125–150[l]	—
Corbicula sandai (*158*)[m]	22	63	120–150[l]	9.3
Corbicula japonica (*149, 158*)[m]	37	38	120–150[l]	8

Table VII (*Continued*)

Classification	Unsaponifiable fraction of fat (%)	Sterol of unsaponifiable fraction (%)	Sterol or m.p. of steryl acetate	$\Delta^{5,7}$-Sterols in mixture (%)
Sanguinolaria olivacea (*161*)	32	52	137–138	2.3
Family Solenidae (razor shell clams)				
Solen gouldi (*158*)	42	53	143–144	5.2
Family Myidae				
Mya arenaria (sand clam) (*138*)	20	—	130–131	—
Class Cephalopoda				
Subclass Dibranchia (Coleoidea)				
Order Sepoidea				
Family Sepiidae				
Sepia officinalis (*94*)	—	—	Cholesterol	—
Order Teuthoidea				
Family Ommastrephidae				
Ommastrefes sloani pacificus (*156*)	—	36	Cholesterol	—
Todarus sagittatus (*170*)	—	—	Cholesterol	—
Order Octopoda				
Family Octopodidae				
Octopus vulgaris (*7, 171*)	—	—	Cholesterol	—

[a] Also considerable quantities of diunsaturated sterols.

[b] Although the data are rather incomplete this species has been included to point to the unusual composition of its sterol mixture.

[c] Poriferasterol, clionasterol, β-sitosterol, $\Delta^{5,7}$-sterols, and others.

[d] Brassicasterol, cholesterol, $\Delta^{5,7}$-sterols, and others.

[e] "Pincsterol."

[f] "Conchasterol."

[g] "Ostreasterol" (chalinasterol).

[h] 24-Methylenecholesterol.

[i] "Pectosterol."

[j] "Shakosterol."

[k] "Meretristerol."

[l] Contains corbisterol.

[m] Freshwater species.

IX. Sterols of Echinoderms

A considerable, instructive body of data has become available during the past decade on the sterols of animals encompassed by this phylum. Representatives of all its five classes and of the majority of their orders have been investigated. Their total number and their representation of various climatic and geographic zones are more than adequate to lend significance to the data so far obtained. Table IX lists the species from which meaningful data have so far been derived. The data include the fat content of the animal, rather vaguely expressed as ether-, acetone-, or benzene-soluble material, the unsaponifiable fraction and its sterol content, and the most characteristic and informative physical properties of the sterol fraction.

The fats of the starfish contain a relatively high amount of unsaponifiable matter, and the highest average, 27%, among the Phanerozonia. The considerable deviations from the average may not be due solely to species differences or seasonal variations but more likely to the methods used in the isolation of the unsaponifiable fraction. The latter contains substantial quantities of batyl alcohol, which is rather water soluble and often incompletely extracted with ether. Such incomplete extraction will in turn influence the sterol content of the unsaponifiable fraction.

The sterols of starfish were among the first animal sterols to be recognized as different from cholesterol. Dorée (*6*) called attention to their peculiar properties, and Kossel (*172*) gave a more detailed description of the sterol of *Asterias rubens*, which he named stellasterol. Many years later it was shown that the typical starfish sterol is a mixture of the monounsaturated stellastenol and the diunsaturated stellasterol (*173*). Such mixtures are exceedingly difficult to separate, and the components of most of them have not yet been obtained in a convincingly uniform state. It has, however, been established beyond doubt that both sterols possess the Δ^7-double bond rather than the more familiar Δ^5-double bond, and that they differ only in the structure of the side chain. Interpretation of evidence derived from ozonolysis of the mixture led to the formulation of stellastenol as (III, R = G) and stellasterol as its 22,23-dehydro-derivative (III, R = H) (*173*).

In the Japanese literature an analogous mixture of sterols derived from other starfish was described almost simultaneously under the name hitodesterol (*20*, *174*). More recently the suggestion has been made that hitodesterol from *Asterias amurensis* (*175*), and *Asterias pectinifera* (*176*) is identical with α-spinasterol, a Δ^7-sterol (III, R = K) long known as the sterol of spinach. It is either accompanied if not replaced by other Δ^7-sterols, such as Δ^7-cholestenol (*177*) (III, R = A), Δ^7-stigmastenol (III, R = J), and a sterol such as (III, R = K) (*178*). Pateriasterol (*179*) from *Pateria* (*Asterina*) *pectinifera* for which a structure had been proposed in disagreement with its optical properties, has since been found to be identical with hitodesterol, i.e., one or more of the Δ^7-sterols (*177*). The name asteriasterol was given to a low-melting unsaponifiable material from *Asterias forbesi* (*180*). Subsequent studies showed that this fraction contained only little sterol and consisted mainly of astrol first isolated from starfish by Kossel (*172*) and subsequently identified as batyl alcohol (*181*).

Table IX includes species typical of all three recognized orders of Asteroids. The basic order of Phanerozonia, which is believed to have given rise to the Spinulosa and Forcipulata (*182*), is particularly well represented by families. Only species from the Australian Ondonasteridae and the deep-sea family of Benthopectinidae are missing from a comprehensive coverage of this order. The important families of the Spinulosa are also adequately covered, and only the Pterasteridae, the cushion stars, and the small borderline family of Ganeriidae, including starfish from antarctic and subantarctic regions, still merit attention. Among the Forcipulata only the large family of Asteriidae has so far been investigated. Data on species from the deep-sea families of Zorioasteridae and Brisingidae, which are possibly related to Ophiuroids, are desirable for a satisfactory completion of the picture.

The most outstanding fact to be derived from Table IX is the ubiquity of Δ^7-sterols in asteroids. The small rotations of the crude and purified products clearly show that such sterols are the principal if not exclusive components of the sterol mixtures so far studied, and it is reasonable to assume that they are characteristic of all asteroids. It remains a challenging problem, however, to elucidate the compositions of the sterol mixtures, to identify all its components, and to search for any connections between them and the classification of asteroids. Such data might for example be helpful in a reappraisal of the Asteriidae, which appears to be a polyphyletic aggregation of genera.

The two orders of the ophiuroids (Table IX) are represented by a reasonably adequate coverage of families. With the exception of some of the basket stars, Euryalae, most of the ophiuroids are rather small, and although quite common, they are difficult to obtain in quantities adequate for a thorough sterol analysis. The rotations of the sterol mixtures clearly indicate the predominant presence of Δ^5-sterols (II). The Δ^7-sterols, which are so characteristic in the starfish, appear to be absent altogether. In the case of *Ophiura sarsi* no Δ^7-sterol was detected in the sterol mixture by means of Fieser's very sensitive color test (*45*). The ophiuroid sterols seem to be rather complex mixtures of Δ^5-sterols, reminiscent of plant sterol mixtures. It is doubtful that any pure sterol has yet been isolated from ophiuroids, but the presence in the mixture of cholesterol, clionasterol, poriferasterol, and stigmasterol has been mentioned (*158*).

Our present knowledge of the sterols of the crinoids is in a similar state of advancement. The living species of this class of echinoderms, which is best known for its fossil record, are all included in one order.

Representatives of its most typical families have been the subject of some preliminary studies. These have shown convincingly that the crinoid sterols are also of the levorotatory, Δ^5-type (II). Beyond this, however, very little is known except for an indication that crinoids may contain one or more sterols not previously encountered in nature (*89*).

Although the holothurians, or sea cucumbers, are the fleshiest and least inorganic of the echinoderms, they also contain the relatively lowest amount of fat. Its average unsaponifiable fraction is also below the usual value for echinoderm fats. Since in addition the sterol content rarely exceeds 10% of the unsaponifiable fraction, relatively large quantities of holothurians are needed for meaningful studies. This is one of the major reasons why the sea cucumbers have not yet been as adequately covered as other classes of echinoderms. Only two of the five orders are represented in Table IX. It is hoped that data on species from the order of the interesting deep-sea holothurians, Elasipoda, and from Molpadonia and Apoda will become available in the near future. All species which have so far been investigated have afforded Δ^7-sterols reminiscent of the starfish sterols, in their low rotations and their color reactions. Their separation into identifiable compounds has not yet been accomplished.

Although the echinoids, or sea urchins, include species such as *Arbacia* and *Echinus*, which have been studied intensely by generations of biologists, our knowledge of their sterols is surprisingly fragmentary. It may be safely stated, however, that the echinoid sterols are of the Δ^5-type (II), and that cholesterol is often if not always a major component. In *Arbacia* the sterol mixture contains at least 70% of cholesterol (*89*). In other sterol mixtures the presence of significant quantities of additional sterols is indicated by the relatively high melting point of the crude steryl acetate mixture. Identification of these sterols and the correlation of their occurrence with the classification and phylogeny of the echinoids remains an interesting problem.

The species of echinoids listed in Table IX have been arranged on the basis of the classification given in Hyman's monograph (*182*). They do not include representatives from the more primitive order of Cidaroidea, the slate-pencil urchins, nor from the interesting and almost extinct order of Cassiduloida.

Over forty years ago Mathews (*183*) called attention to "an important chemical difference between the eggs of the sea urchin and those of the starfish." From color tests he deduced that sea urchin eggs contain cholesterol and that starfish eggs do not. Today we know that this difference does indeed exist, that it is typical also for the entire animals, and that it even divides the entire phylum. If we were to classify the various species of echinoderms on the basis of their sterol content, they would fall within two major groups. The one containing the unusual Δ^7-sterols (III) would include the asteroids and holothurians. The other, bearing the Δ^5-sterols (II) would embrace the ophiuroids, the echinoids, and the crinoids (8). These differences are so characteristic as to merit special consideration.

It does not appear probable that such structural differences are connected with the feeding habits of the animals, and that the sterols are essentially exogenous and reflect the structures of the food sterols. The asteroids, which contain Δ^7-sterols, feed on all kinds of sessile or slow-moving animals and also cadavers, all of which contain mainly Δ^5-sterols. In contrast the other Δ^7-sterol-containing echinoderms, the holothurians, are either plankton feeders like the dendrochirotes or extractors of organic matter from surrounding substrates like the aspidochirotes and species belonging to other orders. Similar feeding habits are also encountered among the Δ^5-sterol-containing echinoderms. The ophiuroids combine ingestion of bottom material with carnivorous feeding. The echinoids are on the whole omnivorous with some, like *Echinus*, leaning toward a carnivorous diet, and others like *Strongylocentrotus*, apparently preferring plant matter, and with certain deep-sea species acting as general scavengers. Planktonic plants and animals are the principal food of the crinoids. If exogenous sterols should play a role in the steroid metabolism of the echinoderms one would have to assume that where necessary the location of the cyclic double bond will be so modified as to appear as Δ^7 in some and as Δ^5 in others. The side chains of these sterols, about which there is so little known at present, might possibly reflect the structure of the exogenous sterols in the degree of their unsaturation and their substitution at C-24.

It also does not appear likely, although not impossible, that the structural differences among the sterols are somehow connected with the mineral metabolism of the echinoderms. The Δ^5-sterol-containing species all appear substantially more calcareous than those containing the Δ^7-sterols. To judge from the very few reliable values now available the organic content of the echinoids (12–20%, average 16%) and crinoids (8–19%, average 15%) are about the same. It is significantly lower than that of asteroids, which ranges from 12% for *Astropecten articulatus* to 64% for the very fleshy *Henricea sanguinolenta*, or that of the holothurians which is in excess of 70%. The Δ^5-sterol-containing ophiuroids, however, are not as calcareous as they appear superficially. Their content of organic matter (19–37%, average 30%) overlaps that of the Δ^7-sterol-containing asteroids.

The author has for some time regarded it more likely that the differences in sterol content reflect some phylogenetic relationship among the echinoderms (*8*). The occurrence of the rather unusual Δ^7-sterols in asteroids and holothurians suggests a closer biochemical relationship between these two than between asteroids and the Δ^5-sterol-containing ophiuroids. These in turn may be regarded as more closely related to the echinoids and possibly also to the crinoids. This chemical evidence furnishes interesting support to a phylogenetic relationship among echinoderms which has been derived from embryological evidence. It has led Fell (*184*) to outline a "phylogenetic tree" (Table VIII) which the recapitulation theory would construct from embryological evidence, if larval forms are regarded as repeating ancestral conditions. Fell, however, found it "impossible to accept the evidence which implies that ophiuroids and echinoids are more closely related to each other than to other classes, and that holothurians and starfish are similarly connected," and that "the recapitulation theory as applied to larval forms leads to a *reductio ad absurdum* in the case of the echinoderms." In the absence of convincing evidence to the contrary, the biochemist, however, cannot regard such groupings as absurd which so nicely agree with his own comparative studies.

TABLE VIII

PHYLOGENETIC TREE OF ECHINODERMS BASED ON EMBRYOLOGICAL AND CHEMICAL EVIDENCE

Class				Type of sterol
Dipleurula	Pluteus	Ophiopluteus	→ Ophiuroidea	Δ^5
		Echinopluteus	→ Echinoidea	Δ^5
	Auricularia	Bipinnaria	→ Asteroidea	Δ^7
		Pentacula	→ Holothuroidea	Δ^7

More recently, Hyman has voiced her support (*182*) of the relationship which is so well based on embryological and chemical evidence. Contrary to the more common belief that the ophiuroids are derived from the asteroids, she holds that the asteroids diverged early from this line of evolution "whereas echinoids and ophiuroids have continued for some distance along the common path," and also that a "closer relationship of ophiuroids to echinoids rather than asteroids is no longer to be doubted." In contrast, however, paleontologists (*185*) have arrived at the conclusion that asteroids and ophiuroids are more closely related to each other, having both of them derived from a

TABLE IX
Sterols of Echinoderms

Classification	Fat content of total (%)	Unsaponifiable fraction of fat (%)	Sterol of unsaponifiable fraction (%)	Rotation $[\alpha]_D$°
Class Asteroidea (starfish)				
Order Phanerozonia				
Family Astropectinidae				
Astropecten articulatus (186)	2.9	43.5	23	Sterol, +6.5
Astropecten polycanthus (134)	7.9	26.5	38.5	Sterol, 0
Astropecten scoparius (20, 134)	4.1	29	42	Sterol, 0
Astropecten aurantiacus (172)				Stellasterol
Psilaster florae (186)	2.6	30	19	Sterol, +1.5
Family Goniopectinidae[a]				
Ctenodiscus crispatus (186)	2.9	23	41	Acetate, +6.2
Family Goniasteridae				
Hippasteria phrygiana (186)	4.3	20	42	Acetate, +6
Family Luididae				
Luidia clathrata	2.3	29.5	27	Acetate, +2
Luidia quinaria (134)	8.2	26	40	Sterol, +3
Family Poraniidae				
Porania insignis (186)	7.8	16	17	Acetate, +3.1
Family Oreasteridae				
Oreaster reticulatus (89)	—	—	—	Acetate, +2
Order Spinulosa				
Family Asterinidae				
Asterina pectinifera (20, 176, 186)	3.0	14	25	Sterol, 0[b]
Pateria minitiata (186)	2.4	20	50	Acetate, +4
Family Solasteridae				
Crossaster papposus (186)	9.0	20	15	Acetate, +5.4
Solaster endeca (186)	9.4	29	15	Acetate, +4
Family Echinasteridae				
Henricea sanguinolenta (186)	12.6	9	15	Acetate, +9
Order Forcipulata				
Family Asteriidae				
Asterias rubens (6, 187)	—	39	—	Sterol, +2
Asterias forbesi (173)	—	—	—	Sterol, +3
Asterias rollestoni (173)	7.6	16	53	Acetate, +4
Coscinasterias acutespina (183)	11.8	39	41	Acetate, +0
Coscinasterias tenuispinus (183)	2.7	40	42	Acetate, +0
Pisaster brevispinus (89)	2.0	21.5	—	Acetate, +2

[a] Porcellanasteridae.

[b] Hitodesterol, α-spinasterol, Δ^7-cholestenol.

TABLE IX (*Continued*)

Classification	Fat content of total (%)	Unsaponifiable fraction of fat (%)	Sterol of unsaponifiable fraction (%)	Rotation $[\alpha]_D$°
Pisaster giganteus (*89*)	9.4	7.2	50	Acetate, 3.8
Pisaster ochraceus (*89*)	5.3	18.5	—	Acetate, +0
Pycnopodia helianthoides (*89*)	5.6	15.5	—	Acetate, +4
Class Ophiuroidea (brittle stars)				
Order Ophiurae (brittle stars)				
Family Ophiactidae				
Ophiopholis aculeata (*89*)	—	—	—	Sterol, −36
Family Ophiocomidae				
Ophiocoma riisei (*89*)	1.0	25	30	Sterol, −40
Family Ophiolepididae				
Ophiura sarsi (*186*)	2.4	14	33	Acetate, −49
Family				
Ophioplocus japonicus (*183*)	2.1	30	35	Δ^5-Sterols[c]
Order Euryalae (basket stars)				
Family Asteronychidae				
Asteronyx loveni (*186*)	2.6	18	39	Acetate, −47
Family Gorgonocephalidae				
Gorgonocephalus arcticus (*186*)	2.3	20	43	Acetate, −47[d]
Gorgonocephalus caryi (*183*)	2.3	30	62	Acetate, −39[e]
Class Crinoidea (sea lilies)				
Family Pentacrinidae				
Cenocrinus asteria (*89*)	2.6	19	29	Acetate, −41
Family Antedonidae				
Antedon rosacea (*89*)	2.6	40	30	Acetate, −40
Family Comasteridae				
Comaster japonicus (*183*)	5.3	17	43	Acetate, −38
Comatula sp. (*186*)	1.5	30	35	Acetate, −47[f]
Class Holothuroidea (sea cucumbers)				
Order Dendrochirota				
Family Cucumariidae				
Cucumaria chronhjelmi (*183*)	2.0	17	8	Sterol, 0
Cucumaria frondosa (*89*)	—	13	5.5	Sterol, 0
Cucumaria quinquesemita (*188*)	—	—	—	Δ^7-Sterol
Thyone briareus (*89*)	1.0	17	14.5	Δ^7-Sterol

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[c] Poriferasterol (II, R = M), clionasterol (II, R = L), probably also β-sitosterol (II, R = J) and stigmasterol (II, R = K).

[d] Some cholesterol.

[e] Possibly some β-sitosterol (II, R = J).

[f] A diunsaturated sterol has been isolated from this species which does not appear to be identical with any known sterol. It has tentatively been called crinosterol, m.p. 138°, $[\alpha]_D$ −47.2°; acetate, m.p. 147°, $[\alpha]_D$ −47.2°; acetate tetrabromide, m.p. 186°; benzoate, m.p. 143°; $[\alpha]_D$ −17.6°.

TABLE IX (*Continued*)

Classification	Fat content of total (%)	Unsaponifiable fraction of fat (%)	Sterol of unsaponifiable fraction (%)	Rotation $[\alpha]_D$°
Order Aspidochirota				
Family Holothuriidae				
Holothuria princepo (96)	—	11	10	Acetate, +4
Stichopus japonicus (99)	2	17	7	Sterol, 0
Class Echinoidea (sea urchins)				
Order Aulodonta[g]				
Family Diadematidae				
Diadema antillarum (96)[h]	—	20	—	Cholesterol
Order Stirodonta				
Family Arbaciidae				
Arbacia punctulata (186)	2	25	42	Cholesterol
Order Camarodonta				
Family Strongylocentrotidae				
Strongylocentrotus pulcherrimus (158)	—	—	—	Cholesterol
Family Toxopneustidae				
Tripneustes esculentus (96)	—	26	—	Cholesterol
Family Echinometridae				
Echinometra subangularis (89)	1	33	43	Cholesterol
Order Clypeastroida				
Family Scutellidae				
Echinarachnius pasma (89)	1	14	42	Cholesterol
Family Clypeastridae				
Clypeaster japonicus (189)	1.7	19	65	Cholesterol

[g] Former order: Diadematoidea.

[h] *Centrechinus.*

common somasteroid ancestor. Whatever the outcome of the controversy will be, it is certain that comparative biochemical evidence will weigh heavily in the final judgment.

X. Sterols of Chordates

The phylum of Chordata includes the very interesting group of animals which bridge the gap between the invertebrates and the vertebrates. With the exception of the fishlike lancelets, *Amphioxus*, they resemble invertebrates in their external appearance, but they share with the vertebrates the possession of a notochord and pharyngeal respiration Unfortunately almost nothing is known about the sterols of these protochordates. Only a few of their most accessible representatives, the sea squirts (Ascidians) of the subphylum Tunicata have been the subject of exploratory studies. Cholesterol has been found to be the primary sterol of *Styela plicata* (*96*), *Phallusia mammilata* (*190*), and *Microcosmus sulcatus* (*195*). It is accompanied by other sterols such as clionasterol and poriferasterol. The Japanese species, *Cynthia roretzi* (*154*), has afforded a sterol mixture containing a $\Delta^{5,7}$-sterol, tentatively identified as 7-dehydrostigmasterol (I, R = K). Poriferasterol and clionasterol were identified among the remaining sterols, but the presence of cholesterol was not ascertained. Preliminary as they are, these observations point to the possibility that species of Tunicata may differ significantly in their sterol content, and that such differences may reveal interesting phylogenetic relationships.

The sterols of vertebrates have been the subject of numerous investigations. In all species has cholesterol been found to be the principal sterol. It is generally accompanied by small amounts of cholestanol (I, R = A), 7-dehydrocholesterol (I, R = A), and Δ^7-cholestenol (III, R = A). In skin sterols, and in particular in those from rats, Δ^7-cholestenol may be present in quantities equal to if not exceeding those of cholesterol (*11*). Large quantities of trimethylsterols, such as lanosterol (IX), are found in certain hair fats, such as wool grease (lanolin).

A Δ^5-sterol quite different from cholesterol and not of the order of C_{27} has been isolated from the poisonous skin secretions of the toad *Bufo vulgaris* and other species. In its properties (m.p. 146°, $[\alpha]_D$ —38°; acetate, m.p. 137°; dihydroderivative, m.p. 140°; acetate, m.p. 138°, $[\alpha]_D$ +119, it closely resembles γ-sitosterol (II, R = L) (*191*). The same sterol, accompanied by from five to twenty times the amount of cholesterol, has also been isolated from toad skins. This interesting observation emphasizes the rather peculiar character of the biochemistry of this primitive vertebrate in which the synthesis of "digitalis-like" heart poisons and the excretion of arginine are prominent features.

XI. Conclusions

The survey of the distribution and structures of animal sterols which was begun fifty years ago remains far from completion. The catalogue of information does not yet include representatives of several phyla, among which the Brachiopoda, the Bryozoa, and the Phoronides are of particular phylogenetic interest. Species of other phyla are represented in numbers still too inadequate to permit drawing conclusions of more than transitory value. The survey has concerned itself almost exclusively with the sterols as they are obtained from animals, rather than their origin. With the exception of some protozoans and insects, very little is known about the origin of sterols in invertebrates. As yet we do not known which invertebrates depend on an exogenous source of sterols like the insects and which are capable of sterol synthesis like the vertebrates. Certain phytophagous insects have been found capable of dealkylating the "plant sterols" of their diet and converting them to cholesterol, the principal sterol found in the adult animal. It is probable that other invertebrates which might depend on exogenous sterols will also modify them to the compounds which are so characteristic of certain classes, orders, and families of sponges, mollusks, and echinoderms regardless of their feeding habits or their geographic origin.

The greatest diversity of sterols is encountered among the most primitive animals, such as sponges and coelenterates. Cholesterol may be found in such primitive species, be they herbivorous sponges feeding on phytoplankton or carnivorous sea anemones. It is, however, neither the most typical nor the predominant sterol of lower invertebrates. At higher levels of evolution other sterols disappear and cholesterol rises to an increasingly dominant position until it has become the only principal sterol of the vertebrate. Once this evolution in the direction of the almost exclusive use of cholesterol had become apparent, the writer (*8*) suggested "that the reduction of a multitude of closely related compounds which seemingly perform closely related functions, to a few compounds if not only one, is a general phenomenon of biochemical evolution." In this evolution cholesterol may be viewed as representing the survival of the fittest sterol (*192, 193*).

The diverse sterols of invertebrates performing similar functions may be regarded as homologous in the biological sense. Many of them are also homologs in the chemical sense since they differ from each other only in the presence of one or more CH_2-units. Thus β-sitosterol is a homolog of cholesterol in both the biological and chemical sense.* In recent years the theory of the biochemical formation of sterols from squalene (VIII–XIII) has been found to be in complete accord with the experimental facts. The familiar homologs of cholesterol must be formed by the attachment of one or two carbon atoms to the final product or one of the intermediates in this transformation sequence. In the case of the biosynthesis of ergosterol from yeast it has already been shown that the addition of a single carbon unit to C-24 constitutes the final step (*194*).

From the viewpoint of biosynthesis, therefore, cholesterol is the primary and simpler sterol, and the alkyl groups at C-24 are secondary features of as yet unknown significance. In primitive invertebrates these additional features are nearly as common as the unsubstituted side chain

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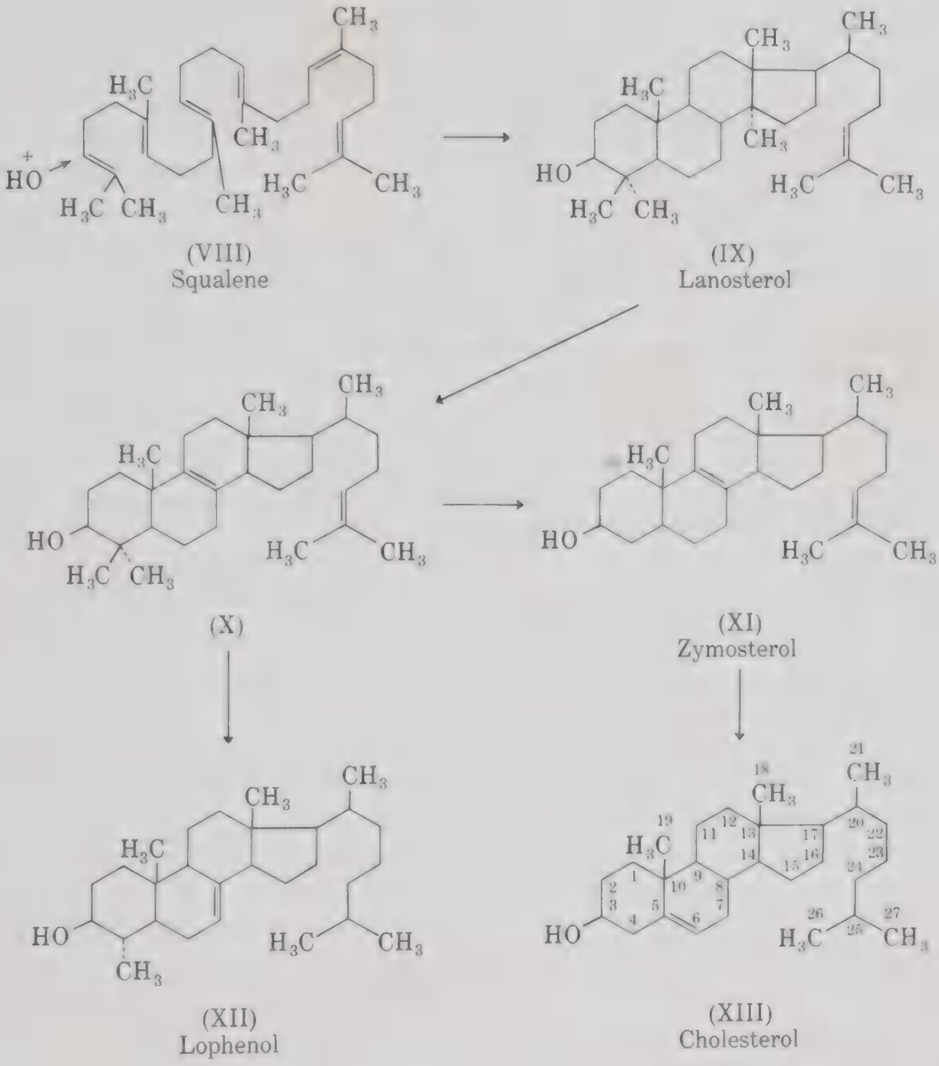
* An analogous connection between homology in the biological and chemical sense is encountered among the fatty acids.



(VIII) Squalene

(IX) Lanosterol

(X)

(XI) Zymosterol

(XII) Lophenol

(XIII) Cholesterol

of cholesterol, but among the vertebrate sterols they have largely if not entirely disappeared. It is for such reasons that the term primitive sterol has two meanings and hence should be used with caution if at all. Thus cholesterol may be viewed as the more primitive sterol when compared with 24-methylenecholesterol. Yet the latter is found as a major sterol only in animals which may be viewed as more primitive than the cholesterol-bearing vertebrates.

In plants the evolution of sterols has taken a different course which has not led to a return to the preferential use of cholesterol. For a long time it had been tacitly assumed that cholesterol was the typical animal sterol and altogether absent from plants. It was shown only rather recently that this is not true and that cholesterol is the principal sterol of certain red algae (*32*). Biosynthetic precursors of cholesterol have also been isolated from plants, such as zymosterol (V, R = B), one of the minor yeast sterols, and quite recently also a methylsterol, lophenol (XII), from a cactus (*57*). The cholesterol skeleton of the twenty-seven carbon atoms of cholesterol is also present in many of the sapogenins and alkaloids found in higher plants.

Nevertheless the vast bulk of plant sterols as presently defined carry substituents at C-24 of the side chain. Primitive plants such as fungi, lichens, and certain algae produce preferentially the C-24 methyl or methylene derivatives of cholesterol. Among the higher plants the C-24 ethyl or ethylidene derivatives are the most conspicuous (*18, 195*). The occurrence in plants of methylsterols substituted at C-24, such as citrostadienol (4α-methyl-24-ethylidene-Δ^7-cholestenol) (*58*), shows that the side chain alkylation may well occur at one or the other of the stages preceding the final step in the cholesterol biosynthesis.

While the vast majority of animal and plant sterols are of the Δ^5-type (II), Δ^7-sterols (III) strikingly predominate in animals belonging to one of the order of sponges, to the most primitive class of mollusks, and to two of the classes of echinoderms. Such sterols are met with also in plants, but their occurrence appears to be more sporadic and less intimately associated with taxonomic features than in animals. An outstanding exception are plants of the family of Cucurbitaceae in which Δ^7-sterols have always been found in major amounts (*18*). The full significance of the preferential use of Δ^7-sterols over Δ^5-sterols still remains to be elucidated, as is the striking predominance of saturated sterols in certain sponges. The solutions to these and other problems, which will lift the comparative biochemistry of sterols from the cataloguing stage to a level of true phylogenetic significance, are to come not only from a broader and more detailed study of animal sterols, but also from a better understanding of the biosynthesis and metabolism of the sterols.

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CHAPTER 3

Lipids: Steroid Metabolism

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I. The Biosynthesis of Steroids

A. STEROL BIOSYNTHESIS

1. *Introduction*

For some time it was believed that plants alone could synthesize sterols, and that the C_{27} compound cholesterol, typical and principal sterol of animal cells, was derived from plant sterols in the diet. This belief became untenable when it was observed in balance studies that animals could excrete more cholesterol than could be accounted for by ingested sterols, and that the rabbit cannot convert dietary plant sterols to its characteristic tissue sterol, cholesterol (*1*).

It now appears that insects require sterols in their diet (*2*); yeasts, molds, higher plants and mammals, and birds can synthesize sterols.

Cholesterol occupies a central position in the biogenesis of steroids insofar as it has been shown to be a precursor of the hormonal steroids and bile acids of higher animals. Furthermore the biosyntheses of the more complex C_{28}, C_{29}, and C_{30} sterols of plants and lower organisms

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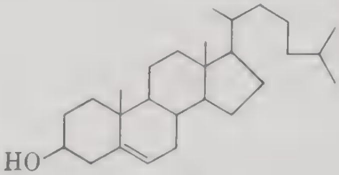

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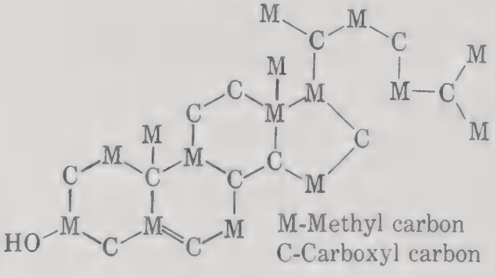

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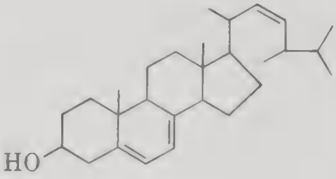


(III)

appear to follow pathways similar to that of cholesterol, deviating only at a relatively late stage. It is thus fortunate that present knowledge of cholesterol biogenesis, based on investigations with animal tissues, plants, yeasts, and molds, is extensive and gaps in this knowledge are being filled in rapidly.

2. *Biosynthesis of Cholesterol*

The biosynthesis of cholesterol will not be considered in detail here since comprehensive reviews are available elsewhere (*3a,b,c*). The following account emphasizes the general nature of reactions involved. Figures 1a and 1b are intended to summarize the present state of knowledge.

The structure of cholesterol (I) gives little indication of the nature of possible precursors, and most of the early schemes for its biosynthesis were of a speculative nature. Evidence that cholesterol may arise from small units was provided by Rittenberg and Schoenheimer (*4*), who found that a high concentration of deuterium is incorporated into stable positions in the cholesterol of mice, the body water of which had been enriched with heavy water. It was subsequently shown (*5*) that acetate may be a major source of the carbon atoms of cholesterol, and when degradation studies revealed the origin of each carbon atom of cholesterol (II) derived biosynthetically from isotopically labeled acetate, it was possible to conclude that the entire cholesterol molecule may be constructed from acetate as the sole source of carbon (*3a*). This work was done with rat liver. Conclusive proof, however, is still lacking that acetate is the sole primary unit for cholesterol biosynthesis in mammalian tissues. It has been shown that this is very probably so for the closely related compound ergosterol (III) synthesized by *Neurospora crassa* (*6*). A mutant strain of this organism requiring acetate as a growth factor was used.

As might be expected, coenzyme A is necessary for the synthesis of cholesterol from acetate.

The distribution pattern of acetate carbons in the isooctyl side chain of cholesterol as revealed by the degradation studies suggested the possibility that cholesterol might be derived from isoprenoid precursors (*7*). This idea was supported by the isolation of labeled squalene (IX) derived from C^{14}-acetate from rat liver and its subsequent transformation by mouse liver into labeled cholesterol (*8*). The C_{30} triterpenoid hydrocarbon squalene had previously been found in the livers of elasmobranch fishes (*9*) and is now known to be widely distributed, occurring among other places in the sebum of animals (*10*). As early as 1926 a biochemical relationship between squalene and cholesterol was suggested and supporting evidence was obtained from experiments in which it was shown that there was an increase in the cholesterol of the livers of rats fed squalene (*11*), but the matter was not pursued further at the time.

The search for the acetate product which condenses was influenced by work in other fields. Thus Bonner and Arreguin (*12*) found that acetate or the branched-chain compound β,β-dimethylacrylate stimulates formation of the polyisoprenoid, rubber, in guayule plants. They suggested that three acetate molecules condense with loss of a carbon atom to form an isoprenoid "building unit," such as dimethylacrylate, which may condense to form rubber. In addition to dimethylacrylate a number of C_5 and C_6 branched-chain acids, which may be synthesized from acetate by plants, have been considered to be cholesterol precursors (*13*). However, the efficiency with which they are converted to cholesterol by liver preparations is low and it was difficult to decide which, if any, was an intermediate. A significant advance was made in 1956 with the isolation of mevalonic acid (β,δ-dihydroxy-β-methylvaleric acid, V) from "brewers' solubles," a product of alcoholic fermentation

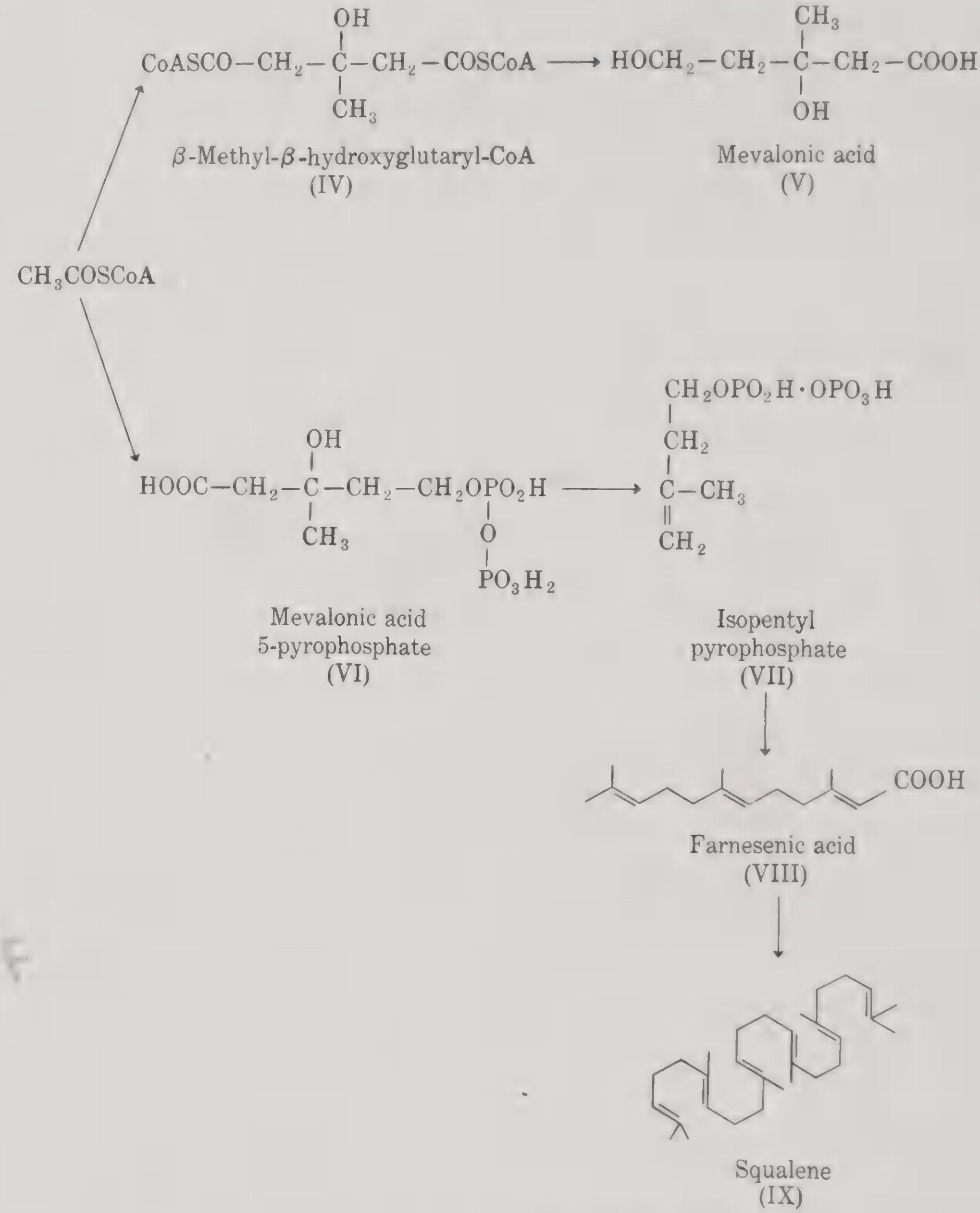

CoASCO–CH₂–C(OH)(CH₃)–CH₂–COSCoA
HOCH₂–CH₂–C(CH₃)(OH)–CH₂–COOH
β-Methyl-β-hydroxyglutaryl-CoA
(IV)
Mevalonic acid
(V)
CH₃COSCoA
HOOC–CH₂–C(OH)(CH₃)–CH₂–CH₂OPO₂H–O–PO₃H₂
CH₂OPO₂H·OPO₃H–CH₂–C(CH₃)=CH₂
Mevalonic acid
5-pyrophosphate
(VI)
Isopentyl
pyrophosphate
(VII)
COOH
Farnesenic acid
(VIII)
Squalene
(IX)

FIG. 1a. Reaction sequence showing substances which may be intermediates in the biosynthesis of squalene from acetate.

(*14*). This substance was first recognized as a factor which could replace acetate in certain strains of lactobacilli. It is also very efficiently converted to cholesterol by rat liver (*15*). When oxygen is excluded, only squalene is formed (*16*). Degradation of squalene prepared from 2-C^{14}-mevalonic acid showed that it contained six atoms of C^{14} per

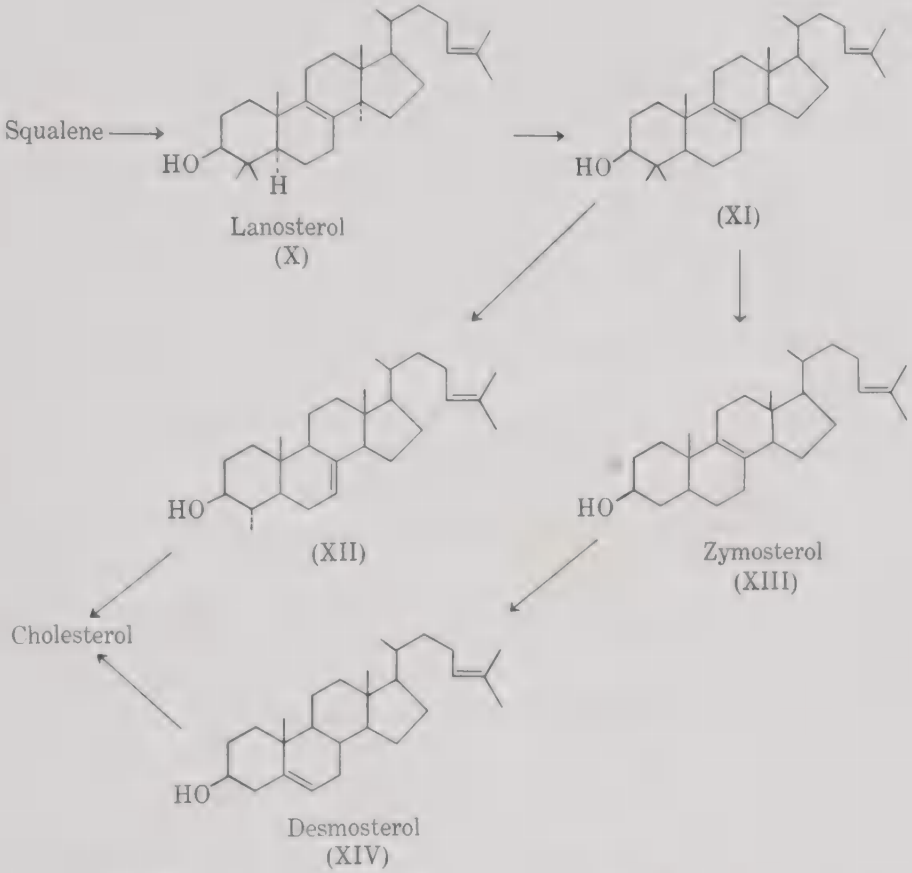


FIG. 1b. Reaction sequence showing substances which may be intermediates in the biosynthesis of cholesterol from squalene.

molecule, supporting the view that mevalonic acid forms an "isoprene unit" six of which condense to form the C_{30} squalene. A similar distribution of isotope was found in cholesterol prepared biosynthetically from 2-C^{14}-mevalonate (*17*). It seems clear from present evidence (*18*) that mevalonic acid can serve as the direct source of the isoprenoid units for the biosynthesis of squalene and cholesterol.

Little is known of the biosynthesis of mevalonic acid other than the facts that it is formed during yeast alcohol fermentation and enzymes have been found in yeast which transform β-hydroxy-β-methylglutaryl coenzyme A (IV) to mevalonic acid. There is, however, no evidence that the coenzyme A derivative is a natural precursor of mevalonic acid. Mevalonic acid formation has not been detected during squalene or sterol biosynthesis by mammalian tissues possibly because of the efficiency with which it is transformed to other substances. If this is indeed the case, the formation of mevalonic acid must be severely rate limiting and may thus be a possible site of control of the whole biosynthetic process by hormones or other factors (*18*).

Steps between mevalonic acid and squalene are not yet clear. The symmetry of the squalene molecule (IX) suggests that it might be formed by a head-to-head union of two C_{15} intermediates, such as farnesenic acid (*19*) (VIII), but satisfactory evidence that this substance is an intermediate is lacking. In a general approach to the problem of intermediates in the biosynthesis of animal and plant sterols by Tschesche and Korte (*20*) a hypothetical C_{10}-hydroxy acid is visualized as condensing with a variety of compounds to give steroids of varying carbon content.

Mevalonic acid-5-pyrophosphate (VI) and a Δ^3-isopentenyl pyrophosphate (VII) have been isolated from yeast and may be intermediates (*21*).

There has been much discussion on the way in which the squalene chain folds and undergoes cyclization (*22*). The accepted folded structure is shown at (IX) and it seems likely that this undergoes cyclization in one enzymically catalyzed step to give lanosterol (X). This substance, found in yeast and wool fat, was formerly regarded as a triterpene since, like squalene, it has 30 carbon atoms. It is now known to possess the steroid tetracyclic structure and thus forms a structural link between the triterpenes and the steroids. It may be noted that the proposed scheme of cyclization (Fig. 1a,b) involves a shift of methyl groups, a rather unusual type of biochemical reaction. Another well-established example of this type of biochemical intramolecular rearrangement is the transformation of *p*-hydroxyphenylpyruvic acid to homogentisic acid. The enzyme which catalyzes the cyclization of squalene has been called squalene-oxidocyclase I since it may be the first to be discovered of a series of enzymes, of general biosynthetic significance, involved in the cyclization of a variety of triterpenes.

Squalene-oxidocyclase I shows features in common with a group of enzymes including nonspecific liver hydroxylase, phenolase, kynurenine hydroxylase, steroid 11β "hydroxylase," and possibly other steroid "hydroxylases" described by Mason as "mixed-function oxidases" (*23*). It has been shown in experiments with O^{18} that reactions catalyzed by these enzymes involve molecular oxygen and not oxygen derived from water, many have been shown to require the presence of a heavy metal, and all the presence of a reducing agent, usually reduced triphosphopyridine nucleotide.

Possible intermediates between lanosterol and cholesterol are 4,4′-dimethylcholestadien-3β-ol (XI) (*24*) and 4α-methylcholestenol (XII) (*25*) isolated from rat tissues, zymosterol (XIII) a yeast sterol also detected in pig liver (*26*), and desmosterol (XIV) found in chicks (*27*).

3. *Sites of Cholesterol Biosynthesis in Animals*

Although much of the work on cholesterol biosynthesis in mammals has involved the liver, many other tissues in a variety of mammals and birds have been found to synthesize this sterol. These include the gonads and adrenals, aorta, blood, intestines, skeletal muscle, and skin. It might be concluded that synthesis of cholesterol is a function of all cells. Nervous tissue, however, is able to synthesize cholesterol only during myelinization.

Investigations with liver cell "fractions" have indicated that the endoplasmic reticulum (microsomes) and the soluble portion of the cell sap are required for cholesterol synthesis (*28a,b*). Nuclei and mitochondria do not appear to be necessary *in vitro* although they may well influence sterol synthesis *in vivo*.

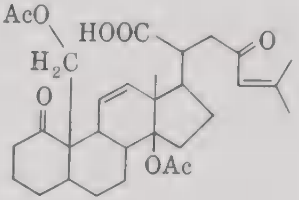
4. *Sterol Biosynthesis in Yeasts and Other Organisms*

Although there is a considerable body of information on the distribution of sterols in living organisms, almost nothing is known of the biosynthesis of these substances in cells other than those of a few species of animals and of yeasts.

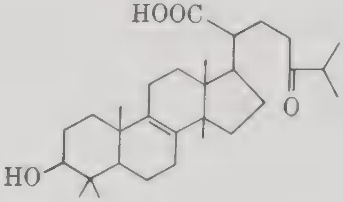
Ergosterol, the characteristic of C_{28} sterol of yeast, has been shown to be formed from acetate (*29*), mevalonic acid (*30*), or squalene (*31*). The additional carbon atom, the methyl group at C-24, is derived from formate, which was shown not to contribute carbon atoms to any other part of the structure (*32*).

An interesting group of 4,4′,14-trimethyl steroids related to lanosterol has been found to have antibiotic properties (*33*). These include helvolic acid (XV) from the flora of sea water obtained near a sewage outlet in Sardinia, eburicoic acid (XVI), and polyporenic acid (XVII) found among products of wood-rotting fungi of the Basidiomycetes. The fungus *Polyporus sulfureus* can also produce eburicoic acid, making use of formate for carbon atom at C-28 (*34*). The absence of an oxygen substituent at C-3 in helvolic acid is remarkable unless the organism by which it is produced has the ability to remove the C-3 oxygen after the steroid is synthesized.

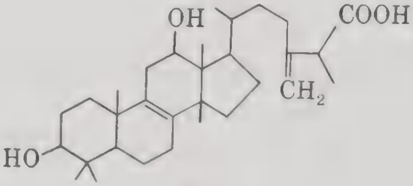
The sterols of marine invertebrates have been extensively studied with particular reference to the occurrence of these substances in the different phyla and classes. Nothing is known of the details of sterol biosynthesis in these animals, but substances postulated as intermediates in cholesterol biosynthesis in higher animals have occasionally been



(Provisional structure)
(XV)



(XVI)



(XVII)

found in their tissues. Thus, for example, 24-dehydrocholesterol (desmosterol, XIV) has been isolated from a barnacle (Entomostraca) (*35*).

Some insight has recently been obtained into the mechanism of the nutritional requirement of insects for sterols (*36*). Larvae of the beetle *Dermestes vulpinus* require cholesterol for growth. It has been found that they can incorporate acetate into squalene but not into lanosterol or cholesterol. Sterol biosynthesis in these insects appears to be blocked at the stage of squalene cyclization. Squalene formed accumulates but is not transformed into sterol.

5. *Conclusions*

It now appears likely that the biosynthesis of sterols is adhering to a general biosynthetic scheme involving isoprenoid units which would lead alternatively to the formation of sterols, terpenes (*37*), carotenoids (*38*), and possibly other substances such as vitamins E and K and chlorophyll (*39*) (Fig. 2). Thus the discovery of mevalonic acid as a growth factor for an organism (lactobacillus) which apparently does

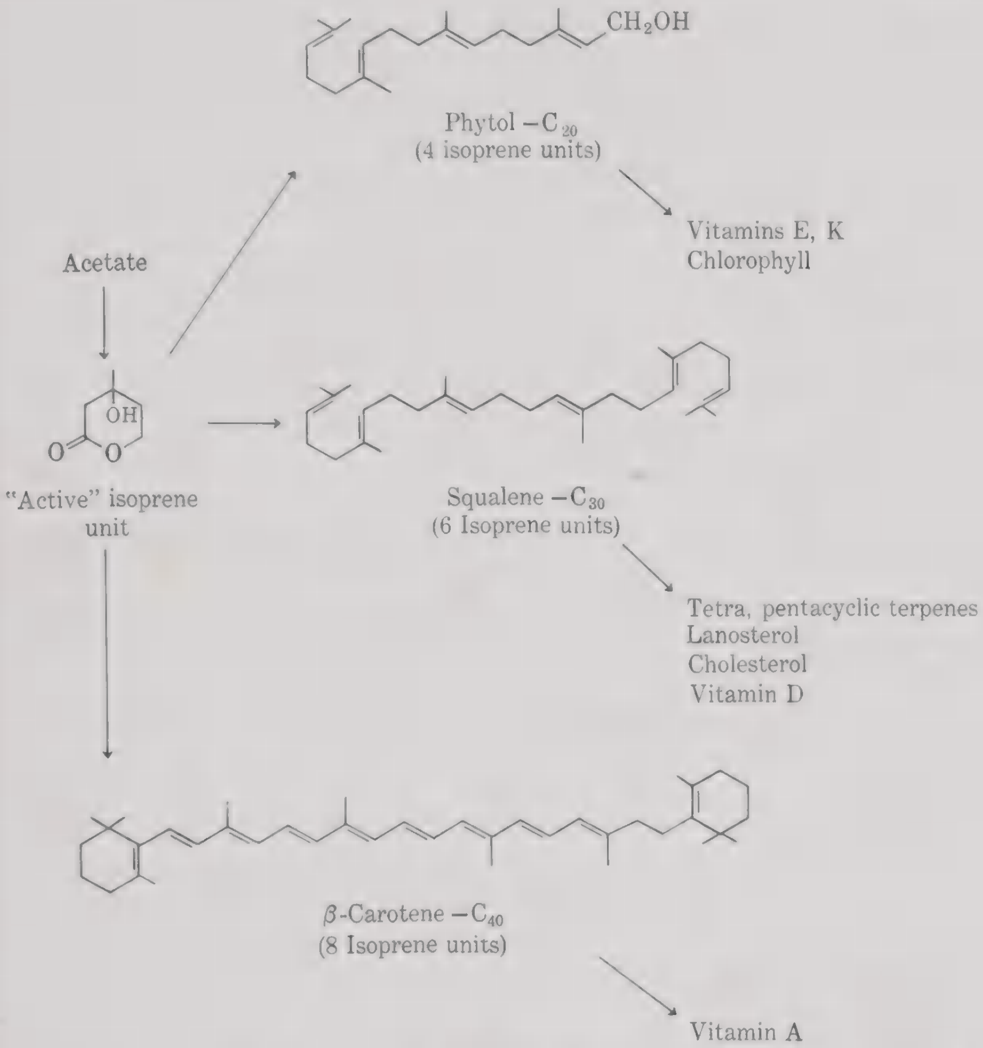


FIG. 2. Formation of cholesterol as part of a general biosynthetic scheme involving mevalonic acid ("active" isoprene unit) as a general precursor. Adapted from Isler and Zeller (*39*).

not synthesize sterols becomes less remarkable. Presumably mevalonic acid is required for the biosynthesis of some essential polyisoprenoid constituent of the organism.

The possibility of alternative pathways cannot, however, be excluded. Although similarities in the biogenesis of β-carotene and squalene exist, the results of Zabin (*40*) might be interpreted as indicating that the mechanism of formation of the typical carotenoid lycopene may be quite unrelated to that of polyisoprenoid substances in animals or that formation of the polymerizing units may differ in different organisms. Zabin isolated C^{14}-lycopene from ripening tomatoes injected several days before with C^{14}-acetate. The distribution of C^{14} observed during degradation of the lycopene indicated that the carotenoid may be formed from identical isoprenoid units but failed to show how the units are built up from acetate in the tomato. Later the same author reported the incorporation of significant amounts of labeled mevalonic acid into lycopene. Obviously more work is required here to confirm the origin of the carbon atoms in the lycopene and that the biosynthesis of this substance is indeed different.

B. Steroid Hormone Biosynthesis

1. *Introduction*

The principal problems of steroid hormone biosynthesis are: (*a*) the chemical nature of the physiologically active steroids secreted by the endocrine glands; and (*b*) the way in which these substances are made by the cells of the glands. These problems have been investigated almost exclusively in the Eutheria or "higher mammals." Virtually nothing is known of steroid hormone biogenesis in other subclasses of mammals or in birds, reptiles, amphibians, and fishes although physiologically active steroids have been found in the tissues or excreta of representatives of some of these classes.

Early knowledge was obtained by the isolation of physiologically active steroids and their metabolites from urine and endocrine tissues, and understandably much of the information was obtained from animals, such as man, the ox, swine, and horse, which provided amounts of urine or tissue large enough for isolation of steroids by classic methods. Interesting species differences soon appeared. For instance estriol (XXIX) appears to be formed by man alone,[*] and certain B ring unsaturated estrogens such as equilin (XXX) by the horse alone. However a wider search with modern micromethods, such as paper chromatography and infrared spectroscopy, for the separation and identification of steroids might reveal that the distribution of these substances is less restricted than appears to be the case. Indeed there is already slight evidence that this is so (Section I,B,3,*b*).

The earlier work provided indirect evidence on the nature of steroid secretions. With the advent of modern micromethods it has been possible to exploit the direct attack on the problem indicated by the work of Vogt (*41*) on the secretion of the adrenal cortex in dogs and other animals in which the adrenal vein was cannulated. As a result, knowledge of the secretion of the adrenal cortex is extensive; much less attention has been paid to the steroids in spermatic vein blood, and almost nothing is known of the steroids that occur in the ovarian secretion. It is for example often assumed that estradiol-17β (XXVII) is the estrogenic hormone in man and some other species without any direct evidence that this substance is actually secreted by the ovary.

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[*] But see Section I,B,3,*b* (page 181).

The second aspect of the problem of steroid hormone biosynthesis, the way in which the hormones are formed in the cells of the endocrine glands, has been intensively studied during the past decade. Experiments with endocrine tissues *in vitro* have been of value in indicating steroid transformations that can occur and in suggesting possible intermediates between the hormones and cholesterol, which appears to be a common precursor. Many of these experiments have been performed with surviving endocrine tissues from the larger domestic animals such as the ox, sheep, and swine, and while it is freely admitted that the *indications* obtained from such experiments must be confirmed by studies of hormone biosynthesis *in vivo*, such studies are not always convenient to perform on large animals. The work of the late R. D. H. Heard on the biosynthesis *in vivo* of steroid hormones by the pregnant mare (Section I,B,3,*b*) is outstanding, and it is most important that such difficult and expensive experiments should be confirmed and extended.

2. *Biosynthesis of Adrenocortical Steroids**

a. The Chemical Nature and Physiological Activity of the Adrenocortical Secretion. Chemical examination (*42*) of the adrenals of oxen and swine revealed the presence of many steroids but gave no indication of which substances are actually secreted by the gland. Some of the substances found may be hormone precursors or metabolites or artifacts formed post mortem or during working up. The solution to this problem was provided by the study of adrenal venous blood based on Vogt's (*41*) technique and on the perfusion studies of Pincus' group using the large (20 gm.) cow adrenal (*43*). The latter work was undertaken originally in order to identify steroids in the adrenocortical secretion, but was subsequently used to obtain information on possible precursors and their chemical transformations in the gland. (Fig. 3.)

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* There is no satisfactory definition of adrenocortical steroids. These steroids are usually regarded as 21-carbon compounds having oxygen substituents at C-3, C-20, and C-21 and sometimes in other positions. There is, however, direct or indirect evidence that the adrenal cortex also produces C_{19} steroids (adrenal "androgens") and C_{18} steroids (estrogens). Attempts at classification are complicated by recent observations that under some circumstances the adrenal cortex will secrete 21-deoxy steroids and that the testes may under certain circumstances oxidize steroids in the C-21 position.

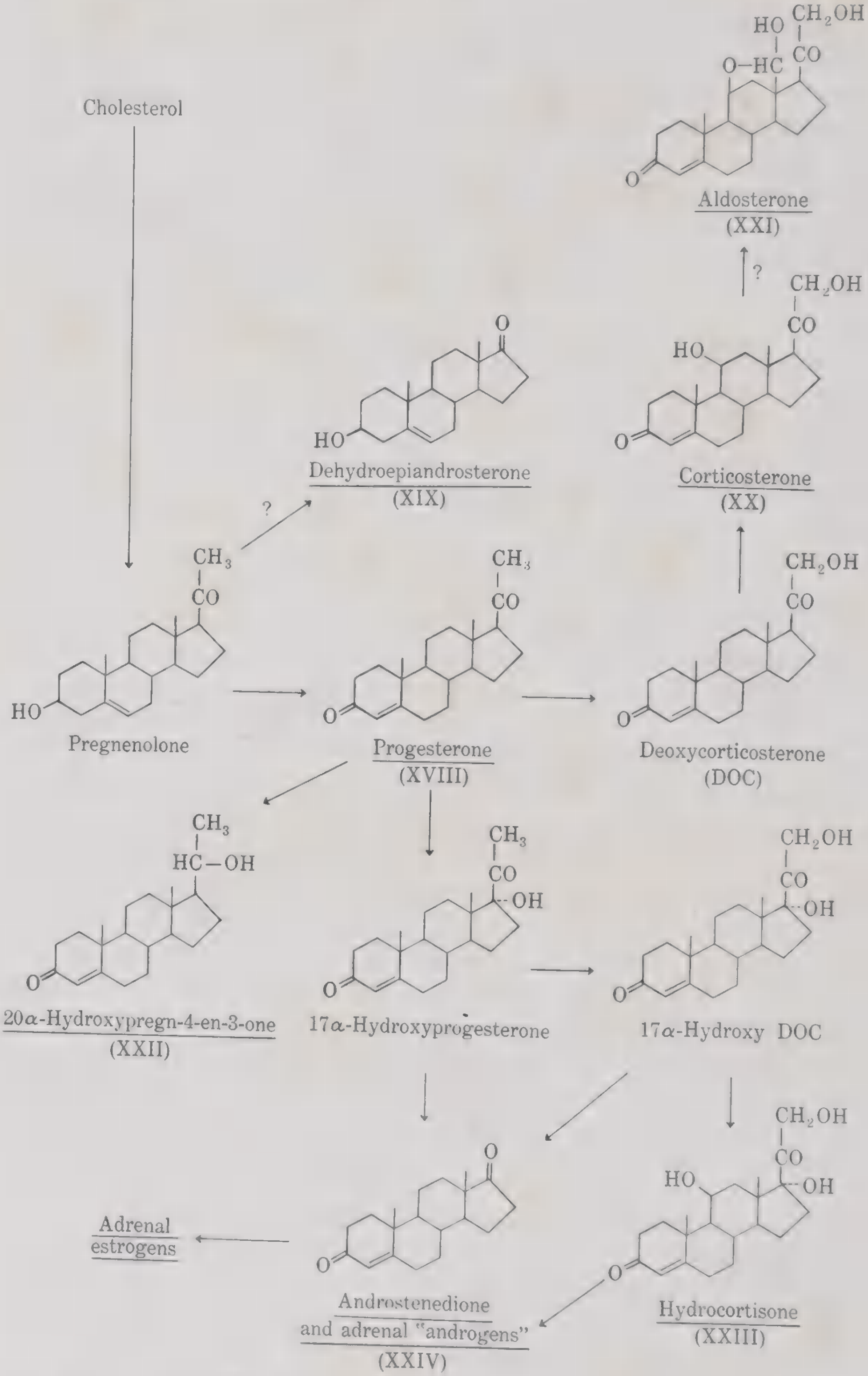

FIG. 3. Scheme for the biosynthesis of steroids in the adrenal cortex.

The result of these and other investigations indicate that C_{21} steroids having oxygen substituents at C-11 and C-21 are characteristic of the adrenal cortex. The principal substances of this nature present in the adrenal venous blood of most species examined are hydrocortisone (XIII), corticosterone (XX), and aldosterone (XXI). The last is present in very much smaller concentrations than the first two, possibly amounting to less than 1% of the total corticosteroid in the adrenal venous blood of dogs; nevertheless it is of major physiological importance (*44–46*).

Administration of any one of these substances will reverse the widespread effects of adrenalectomy which ultimately result in death. They influence many tissues and almost every aspect of intermediate metabolism, and it is unlikely that their biological activities are fully known. A very useful summary of the voluminous literature on this aspect of endocrinology is provided by Chester Jones (*47*). Each of these substances shows qualitatively the same effect on carbohydrate and electrolyte metabolism in test animals, but they differ remarkably in their quantitative effects. Thus, hydrocortisone and corticosterone are predominantly "glucocorticoids," and aldosterone is by far the most active substance influencing electrolyte and water metabolism.

b. The Proportions of Hydrocortisone and Corticosterone Formed. No explanation can be offered for the occurrence in many species of two hormones, hydrocortisone and corticosterone, of apparently similar qualitative and quantitative biological activity. It is of course quite possible that important differences in the biological activities of these two hormones remain to be found. It is notable, however, that some species, including the rat, rabbit, and mouse, seem able to dispense with hydrocortisone.

The relative proportions of hydrocortisone and corticosterone are reported to vary in different species (Table I). These results, however, were obtained with relatively few individuals in any one species, and there is evidence of considerable individual variation within one species. Thus the hydrocortisone:corticosterone ratio has been reported to vary from 10:1 to 1:1 in man and this may be related to the effect of endogenous corticotropin. In dogs selected at random, ratios ranging from 20:1 to 1.2:1 have been reported from one laboratory (*50*). Prolonged administration of corticotropin will induce secretion of hydrocortisone in the rabbit which does not normally secrete this substance in detectable amounts (*51*). Sex may influence the type of hormone secreted since the steer appears to produce corticosterone also, but no hydrocortisone, whereas the cow secretes both hormones as judged by adrenal perfusion experiments (*54*).

c. Trace Amounts of Adrenocortical Steroids. So far only the C_{21} steroids present in major amount in the adrenocortical secretion have been considered. Species differences have been reported in the corticosteroids present in adrenal venous blood in trace amounts (Table II). These differences may, however, be more apparent than real. The differences observed may depend on various factors including techniques used, the size of blood samples available, and the state of stimulation of the adrenal cortex by corticotropin. Moreover, since the substances are present in trace amounts, they are usually detected by color tests and "spot reactions" applied to paper chromatograms, and their identities cannot be considered to be well established.

TABLE I
RATIO OF HYDROCORTISONE: CORTICOSTERONE IN ADRENAL VENOUS BLOOD OF DIFFERENT SPECIES

Species	Ratio	References
Monkey	20	(*48*)
Man	1–10	(*49*)
Sheep	15–20	(*48*)
Ox	1	(*48*)
Dog	1.2–20	(*48, 50*)
Cat	4–6	(*48*)
Ferret	1.5	(*48*)
Rabbit	<0.05	(*48, 51*)
Rat	<0.05	(*48*)
Mouse	0	(*52, 53*)

d. The Rate of Secretion of Adrenocortical Steroids. This has been studied in a few different species. A striking feature of the results is the wide variation in rate found in one species. Thus in the dog some workers (*42, 48, 61, 62*) have found rates varying from 1000 to 3600 μg. per gram of gland per hour where others (*63, 64*) found lower rates (120–700 μg. per gram of gland per hour). Hechter's group (*50*) found values ranging from 132 to 1022 μg. per gram of gland per hour in samples drawn for 1 hour from the adrenal vein of eleven normal dogs, selected at random, after they had recovered from the anesthetic given for cannulation of the vein. It has been observed in the ox, calf (*65*), man (*66*), and rat (*67*) that administered corticotropin causes an increased rate of blood flow through the adrenal gland.

The view that the amount of blood circulating through the gland influences the corticosteroid output is supported by experiments in which the method of perfusion of the cow adrenal was varied (*54*). This gland is provided with numerous small arteries and usually one large vein. The method developed by Hechter's group involves cannulation of the vein through which the perfusing blood enters and laceration of the cortex for its outlet. With this procedure and corticotropin in the blood, a rate of corticosteroid secretion of 387 μg. per gram of gland per hour was achieved, whereas the rate was only 60 μg. per gram of gland per hour when perfusion was in the reverse direction, in through arteries and out through the vein. The blood flow is slower in the latter procedure. Furthermore, it was shown (68) by dilution of the blood with Krebs-Ringer solution that corticosteroid output is a direct function of the amount of blood in the system. Since the blood may be substituted by cow plasma but not by albumin solution, it would appear that the blood contains substances that influence the secretion of corticosteroids in response to corticotropin. These interesting observations should be confirmed and investigated in other species since they may reveal and help to explain fundamental differ-

TABLE II

"CORTICOSTEROID-LIKE"[a] SUBSTANCES PRESENT IN ADRENAL VENOUS BLOOD IN TRACE AMOUNTS

Species	Substance	References
Cat	Cortisone and 2 substances more polar than hydrocortisone	*(48, 55)*
Dog	Cortisone, 11-dehydrocorticosterone, and 2 substances more polar than hydrocortisone	*(48)*
	17-Hydroxy-11-deoxycorticosterone	*(44, 55)*
	11-Deoxycorticosterone	*(45)*
Man	Cortisone, 11β-hydroxyandrost-4-ene-3,17-dione, 17α-hydroxyprogesterone, dehydroepiandrosterone, androst-4-ene-3,17-dione, progesterone;	*(56)*
	11-Deoxycorticosterone not found	*(57)*
	17α-Hydroxy-11-deoxycorticosterone	*(58)*
Monkey	Cortisone	*(48)*
Mouse	11β-Hydroxytandrost-4-ene-3,17-dione	*(52)*
Ox	Progesterone, 20α- (young calf only), and 20β-hydroxypregn-4-en-3-one (adult animal);	*(59,60)*
	Dehydroepiandrosterone, androst-4-ene-3,17-dione, and androsterone not found	*(59,60)*
Sheep	Corticosterone, cortisone, and 1 substance more polar than hydrocortisone	*(48)*

[a] This term is used since the majority of steroids named in this table have not been identified with certainty. Aldosterone, which might be regarded as a trace steroid in adrenal venous blood, is not included in this list.

ences in the biogenesis of adrenocortical steroids in response to corticotropin in ruminants and nonruminants. In this connection it may be significant that the ruminant adrenal glands contain relatively little lipid (Table III).

TABLE III
THE LIPID CONTENT OF ADRENAL GLANDS[a]

Substance	Man[b]	Ox[b]	
		Cortex	Medulla
Lipid	20 (5–30)	6.3	5.6
Phospholipid	3.7	3.1	2.5
Total sterol	10 (2.6–15.0)	0.26	0.35
Ester (as % total sterol)	80–90	10	15–31

[a] Adapted from R. P. Cook, *in* "Cholesterol" (R. P. Cook, ed.), pp. 145–180. Academic Press, New York, 1958.

[b] Results in grams per 100 gm. fresh tissue.

The chemical results are supported by the histological appearance of these glands in which the fasciculata-reticularis zone forms a single zone of cells poor in lipid and having a relatively small cytoplasmic volume, described by Symington (*69*) as "compact" cells. By contrast the adrenals from the great majority of many nonruminant species examined contain much more lipid, especially in the large cells found in the fasciculata zone and described as "clear" cells (*69*). Under the stimulus of endogenous or administered corticotropin the "clear" cells in man and the guinea pig at least are transformed into those of the "compact" type. The "compact" cells in man have been shown (*70*) by electron microscopy to possess microvilli running out into the intercellular spaces in a way which suggests that they may be involved in secretory processes. The ruminant adrenal cortex does not contain "clear" cells and exhibits no changes in cell type in response to stress or corticotropin administration. It is tempting to speculate that in the nonruminants the lipid-laden "clear" cells of the fasciculata zone function as stores of adrenocortical steroid precursor drawn upon under the influence of corticotropin, whereas in the ruminants some factor carried in the blood takes the place of the stored substance.

e. Sex Hormones and Related Substances Secreted by the Adrenal Cortex. In addition to the characteristic C_{21} steroids, the adrenal cortex also possesses the ability to elaborate steroid sex hormones. Progesterone (XVIII), androst-4-ene-3,17-dione (XXIV), and estrone (XXVI) have been found in extracts of cattle adrenal glands (*41*). Analysis of adrenal venous blood has confirmed the presence of progesterone in the secretion of the adrenal gland in man, oxen, sheep, and swine (*56*, *59*).

Androstenedione and dehydroepiandrosterone (DHA) (XIX) have also been detected in the adrenal venous blood of man (*71*), but not of cattle (*56*). Further indirect evidence for the production of adrenal androgens and estrogens in man is provided by the virilizing or feminizing effects observed in some patients with adrenal tumors. Moreover, estrone, estradiol-17β, and estriol have been detected and measured by reliable modern methods in the urine of gonadectomized human subjects (*71a*). There is also evidence that 18-hydroxyestrone, found in the urine of pregnant women, may be of adrenal origin (*72*).

A curious and unexplained observation has recently been made by Short and his colleagues (*60*). They found 20α-hydroxypregn-4-en-3-one (XXII) in higher amounts in the adrenal venous than in the peripheral blood of young calves. This substance has not previously been described as an adrenocortical steroid, but it has been isolated from a number of other tissues in man, the sheep, and the rat, where it has been regarded as a metabolite of progesterone. It appeared in the adrenal venous blood in increasing amounts up to the twenty-third day of life and thereafter decreased. The presence of this steroid was quite unexpected since the 20β-epimer is the major compound of this type present in the adrenal venous blood of adult cattle. The only biological activity of the 20α-epimer known at present is that of a weak progestational agent.

f. The Adrenocortical Hormones in Classes of Animals Other Than the Eutheria. As stated in the introduction to this section, virtually nothing is known of steroid hormone biogenesis in subclasses and classes of animals other than Eutheria. It is not clear at present whether tissue homologous to the eutherian adrenal cortex is present in all vertebrates. The name "interrenal" has been suggested for the organ in certain elsamobranch fishes which appears to correspond to the adrenal cortex in higher animals. The interrenal is rich in lipid (*73*) and, where its surgical removal is possible, disturbances in the electrolyte metabolism of the fish may follow (*74*). Attempts to use crude extracts of elasmobranch interrenals to prevent the appearance of adrenal insufficiency failed in the case of adrenalectomized cats (*75*) but were claimed to be successful in the case of young rats (*76*).

The more common teleost fishes have interrenal tissue distributed about the cardinal veins. In the eel this tissue appears to atrophy after hypophysectomy and regenerate after administration of mammalian corticotropin, and to show changes in ascorbic acid content reminiscent of those observed in mammalian adrenals (*77*).

A hydrocortisone-like substance, showing the reactions of the dihydroxyacetone side chain at C-17 has been detected in the plasma of salmon, and it has been reported (*78*) that salmon migrating to the sea have a higher concentration of this substance in the plasma. The latter observation might possibly be related to changes in metabolism of the fish in preparation for entering a more saline medium. Cortisol has also been tentatively identified in the plasma of carp by paper chromatography. It was estimated that plasma of the male fish contained 24 μg. "hydrocortisone" per 100 ml. and that the female contained twice as much (*79*).

Thus there are a few suggestions that fish may elaborate adrenocortical steroids, but good evidence for this is not available at present.

Similar evidence based largely on the observed effect of hypophysectomy or adrenalectomy is available for amphibians, reptiles, and birds, but facts on the nature of the adrenocortical secretion are lacking (*74*). Grant and Griffiths (unpublished results) have shown that the adrenals of the domestic fowl possess enzymes that catalyze hydroxylation of progesterone and DOC at the 11-, 17-, and 21-positions.

The hypothesis has been advanced that the prime necessity for the evolution of vertebrates was ionic regulation at the kidney level, possibly by adrenocortical hormones. Vital processes are frequently found to be regulated by two opposing factors, for example, the control of blood sugar by glucagon and insulin. It would therefore not be surprising to find that ionic regulation is achieved by two factors: aldosterone promoting sodium retention, and a hitherto unknown factor promoting sodium excretion. There is indirect evidence for the functioning of such a factor in human subjects with certain types of adrenal tumors who have a craving for salt, and strong support for this idea has recently been obtained with the isolation of $3\beta,16\alpha$-dihydroxy-5α-pregnan-20-one from hog adrenals (*80*).* This substance promotes sodium excretion in the rat. Its structure is unlike the known neutral steroid hormones, which possess the Δ^4-3-oxo grouping, and suggests the possibility that an even more active "sodium excreting" steroid remains to be discovered. The 16α-hydroxyallopregnanolone has not so far been found in the adrenal secretion and thus cannot be described as a hormone.

3. *Biosynthesis of Steroid Sex Hormones*

a. Introduction. Apart from information on occurrence in tissues and body fluids of those steroids which influence reproductive organs, little is known about the comparative aspects of the biogenesis of the so-called steroid sex hormones.

The biosynthesis of these substances is not confined to the gonads but may also take place in the adrenal cortex and placenta.

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* The authors have been unable to confirm this observation.

Testosterone has been found in the spermatic vein blood of man (*81*) and the dog (*82*), and progesterone in the ovarian vein blood of the ewe (*83*) and the goat (*84*). Progesterone and the androgen dehydroepiandrosterone have been found in adrenal venous blood in man and the ox (*56, 59, 60*). Otherwise evidence for the elaboration of steroid sex hormones by these tissues is indirect. In no case has the presence of an estrogenic steroid been demonstrated in the blood draining from an endocrine gland.

b. Biosynthesis of Estrogenic Steroids. The principal known estrogenic steroids excreted by man are estrone (XXVI), estradiol-17β (XXVII), and estriol (XXIX). The recently isolated 16α-hydroxyestrone (XXVIII) (*85*) may be a urinary estrogenic steroid of considerable quantitative importance. A variety of other estrogenic steroids have also been found in minor amounts in the urine of pregnant women (*86*).

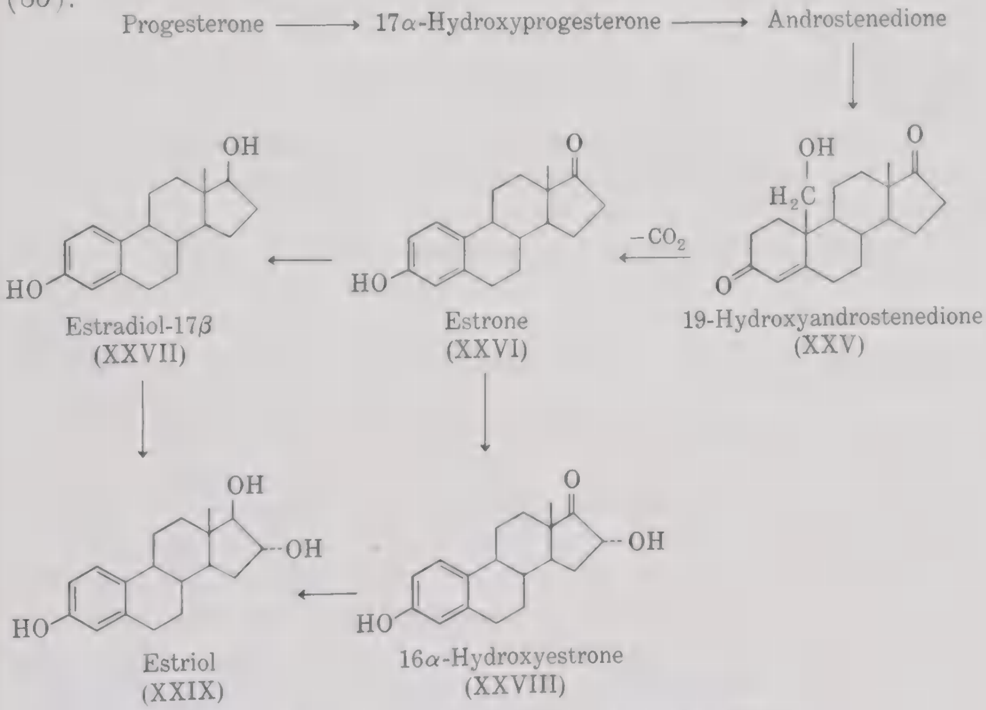
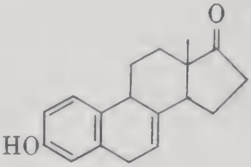


FIG. 4. Scheme for the biosynthesis of estrogenic steroids.

Estriol is usually regarded as being formed exclusively in man, but it has recently been reported in the urine of a dog given estradiol-17β (*87*), among the products of incubating rat liver with estrone (*88*), in the blood of a rat infused with C^{14}-estrone (*89*), and in the ovaries of laying hens (*90*). In no case, however, has crystalline estriol been isolated and thoroughly characterized.

Estrogens excreted in the urine by the horse have been studied extensively (*91*). The principal substances present are estrone, equilin (XXX), and equilenin (XXXI). Other related substances are excreted in minor amount. It is remarkable that estrogens are present in astonishingly large amounts in the genital glands of certain male mammals.



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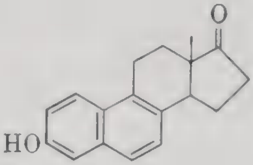


(XXXI)

The richest known source is the testis of the horse, and stallion urine actually contains more estrogenic material than that of pregnant mares. The B ring unsaturated estrogenic steroids have so far been found only in the horse. A trace of an equilenin-like substance, found in a feminizing adrenal tumor removed from a man, unfortunately could not be fully identified for lack of material (*92*).

Estradiol-17α which has not been found in man has been isolated or detected in the urine of horses, rabbits, goats, young calves, and pregnant cows.

It is remarkable that estrone and estriol occur in plants (*93*). Pure crystalline estrone has been isolated from palm kernels and estriol from willow flowers.

Heard (*94*) has studied the biosynthesis of estrogenic steroids from C^{14}-labeled precursors in the pregnant mare *in vivo* and observed that acetate is incorporated into the three principal estrogens of this species. More acetate is incorporated into estrone than into the B ring unsaturated estrogens, suggesting that acetate is utilized in the synthesis of the saturated alicyclic part of the molecule only and that estrone is not a precursor of equilin and equilenin. The utilization of acetate in the synthesis of estrone was ten times greater than in the synthesis of maternal blood cholesterol, and from this it was concluded that cholesterol from the maternal side is unlikely to be a precursor of estrogen. Cholesterol from the fetal side of the placenta contained much more radioactivity and might act as precursor of estrogens in the fetus. Unfortunately in these investigations insufficient consideration was given to the way in which the specific activity of the estrogens and their precursors vary with time, nor was account taken of the fact that the cholesterol probably occurs in metabolic pools which may not be in equilibrium with one another so that, whereas the blood cholesterol may not be significantly labeled, cholesterol at the site of estrogens synthesis may be labeled and may in fact be the precursor of the estrogens formed. Other workers (*95*, *96*) have shown that various preparations of ovaries from dogs, cats, and human subjects could incorporate acetate into cholesterol, estradiol-17β, and estrone, and the conversion of 4-C^{14}-cholesterol to 4-C^{14}-estrone in a pregnant woman has been demonstrated (*96*).

One may conclude that in the dog, cat, horse, and man acetate and cholesterol may act as precursors of estrone formed in the ovary and possibly also in the case of the adrenal glands, placenta, and testes. From *in vitro* studies it has been possible to construct a plausible scheme for the biosynthesis of steroid estrogens from progesterone (Fig. 4) which may be derived from acetate via cholesterol (*97*). It is not known whether such a scheme does in fact operate or if it represents the only biosynthetic pathway for the formation of steroid estrogens. Most of the individual reactions have been demonstrated by means of endocrine tissues (including the placenta) obtained from man and the ox. It is interesting to note that whereas cow placenta, follicular fluid, and adrenal tissue were able to transform 19-hydroxyandrost-4-ene-3,17-dione into estrone, rabbit placenta was inactive (*98*).

c. Biosynthesis of Steroid Sex Hormones in the Placenta and Adrenal Cortex. There is evidence that progesterone, androgens, and estrogens may be elaborated by the placenta and adrenals in addition to the gonads.

Since the ovaries may be removed from the pregnant mare, cow, ewe, bitch, and woman without producing abortion it has been assumed that the placentas of these species produce progesterone. In support of this it has been shown that extracts of cow placenta produce progestational changes in the rabbit endometrium (*99*). It has not, however, been possible to repeat this observation consistently (*100*), and Short (*101*) using a sensitive chemical method found progesterone in the placenta of the mare, but not in the cow, ewe, goat, or sow. The placenta of human subjects is known to be a rich source of progesterone and contains enzymes which catalyze the formation of progesterone from pregnenolone (*102*). These observations suggest the existence of a wide species difference in endocrine mechanisms responsible for the maintenance of pregnancy.

Although it is probable that the placenta can elaborate estrogens, no comparative studies have been made.

Biosynthesis of sex hormones in the adrenal cortex was referred to in Section I,B,2,*e*.

4. *Conclusions*

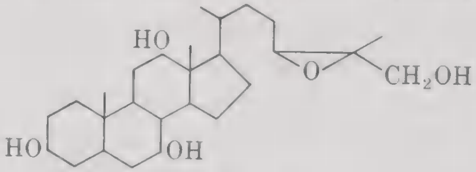
Biosynthesis of the steroid hormones in the adrenal cortex has been most intensively studied. Available evidence indicates that in higher animals the adrenocortical steroids may be derived from acetate or cholesterol by reactions which do not differ significantly in the different species studied. The failure of a few small rodents to secrete hydrocortisone is unexplained. The production of adrenocortical hormones in classes of animals other than the Eutheria is not well established. The investigation of this matter in animals obliged to conserve their body water and in fishes moving from fresh to salt water should prove an interesting and fruitful field of study.

The state of knowledge of estrogen biosynthesis in any animal is particularly unsatisfactory. A search for C-16 oxygen-substituted estrogens in species other than man and for the B-ring unsaturated compounds in species other than the horse would be timely.

C. BIOSYNTHESIS AND TRANSFORMATION OF THE BILE SALTS

1. *Introduction*

Bile salts are either the sulfate esters of steroid alcohols having 27 to 29 carbon atoms, or more commonly hydroxy steroid carboxylic acids having 24, 27, 28, and possibly 29 carbon atoms conjugated with taurine or, in the case of the C_{24} compounds, with glycine. Scymnol (XXXII), found in some elasmobranch fishes, is typical of the steroid bile alcohols, and cholic acid (XXXIV) appears to be the most widely distributed bile acid in vertebrates and is a major constituent of mammalian bile. The C_{24} bile acids are characteristic of the higher vertebrates. The many bile alcohols and acids which occur naturally have been collected in comprehensive tables by Haslewood (*103, 104*). These may be brought up to date by the inclusion of 3α,6α,7β- and 3α,6β,7α-trihydroxycholanic acids recently found in rat bile (*105*).



(XXXII)

Several reviews of this subject are available (*106–108*).

2. *Formation of Bile Alcohols and Acids*

Nothing is known of the formation of bile alcohols. There is good evidence that the C_{24} bile acids are formed from cholesterol. Thus

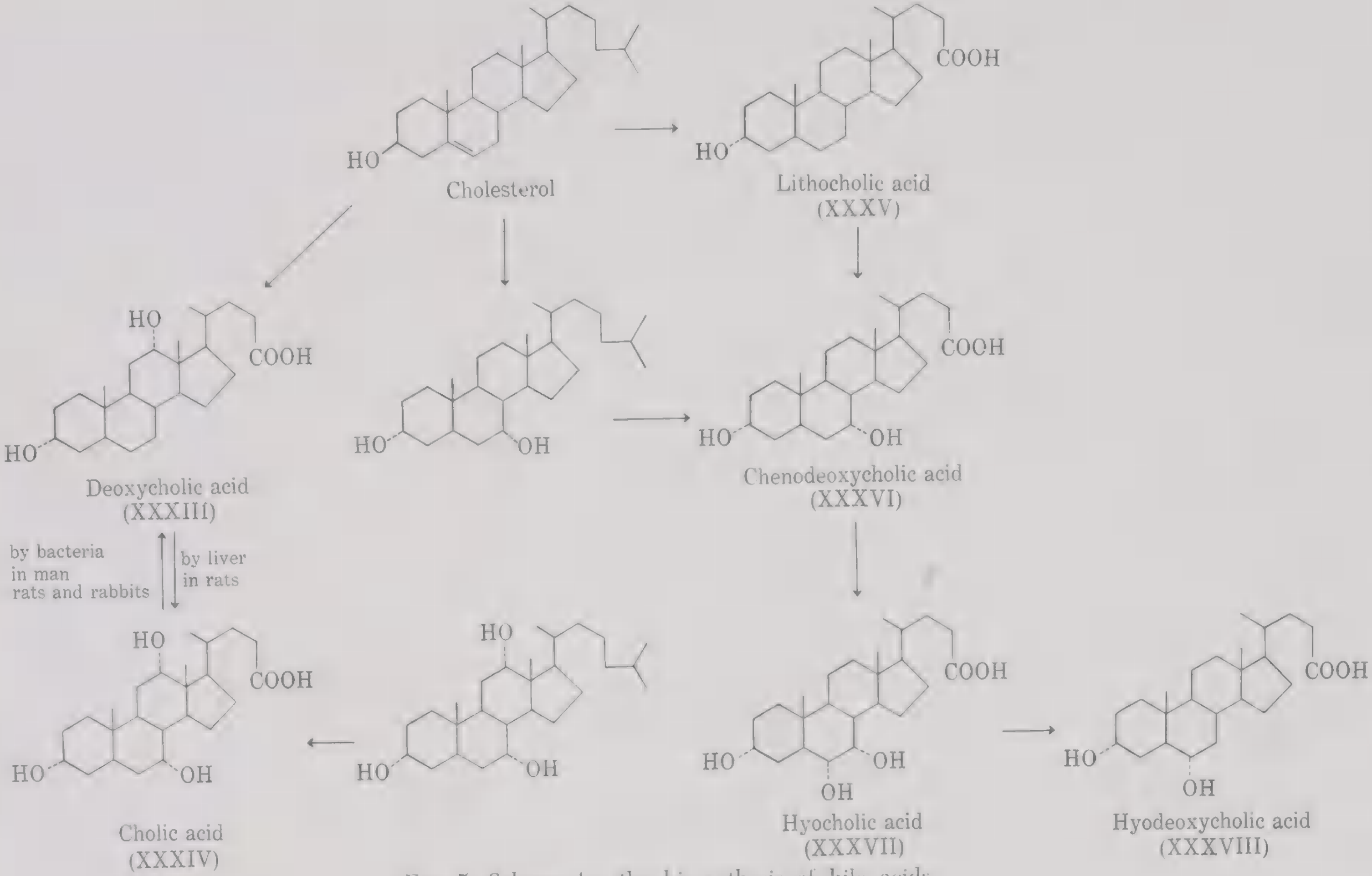


FIG. 5. Scheme for the biosynthesis of bile acids.

H^2-labeled cholesterol injected into a dog is transformed into labeled cholic acid and the quantitative importance of this pathway of cholesterol metabolism has been demonstrated in the rat (*109*). In man, however, isotope studies indicate that injected cholesterol is incorporated more into fecal sterol than into the fecal bile acid fraction (*110*).

A tentative scheme for the biosynthesis of bile acids is shown in Fig. 5. Hydroxylation of the cholesterol rings apparently must occur before side-chain oxidation. An exception to this is the 7α-hydroxylation of deoxycholic acid (XXXIII) which appears to be a reaction specific for the rat—it does not occur in rabbit nor in man (*111*). Until recently bile acid formation in the pig was regarded as unique among mammals in so far as this species effected hydroxylation at the 6α-position. The main bile acids of the pig include hyodeoxycholic acid (XXXVIII) and hyocholic acid (XXXVII) (*112*). Bile acids with 6β-hydroxy substituents, however, have recently been isolated from rat bile (*105*).

3. *Conjugation of Bile Acids*

It has been shown (*113*) that the conjugation of bile acids involves the intervention of coenzyme A, thus

$$\text{Cholic acid} + \text{ATP} + \text{CoA} \rightarrow \text{Cholyl CoA} + \text{AMP} + \text{PP}$$

The enzyme system present in liver cell microsomes shows specificity toward the amino acid involved. In presence of taurine and glycine rabbit liver preparations give glycocholate only; chicken liver, taurocholate; and rat liver, both bile salts. Glycine conjugation has been observed only in mammalian species (*112*). Conjugated bile acids have not been found in feces, bacteria of the large intestine being able to effect hydrolysis (*114*).

4. *Action of Intestinal Bacteria*

The study of the formation of bile acids is complicated by the fact that these substances are transformed by intestinal bacteria. Thus although cholic and chenodeoxycholic acids (XXXVI) account for over 90% of the bile acids in rat bile many more compounds appear in the feces (*106*). One of the main transformations brought about by intestinal bacteria is removal of the hydroxyl at C-7 to give deoxycholic acid. When germ-free rats (*115*) or animals given antibiotics (*116*) are used, this reaction does not occur and the feces contain the same bile salts as the bile. Deoxycholic acid thus formed in the rat may be partially absorbed from the intestine and can undergo rehydroxylation at the 7α-position in the liver. In the case of the rabbit, however, deoxycholic acid formed by the intestinal bacteria is not rehydroxylated

(*117*). This results in the appearance of considerable quantities if conjugated deoxycholic acid in rabbit gall bladder bile, which might be thought to have been formed in the liver. However, when a bile fistula is established in the rabbit the deoxycholic acid disappears from the fistula bile and is replaced by cholic acid. Investigations with animals having bile fistulas have their own complications. When the enterohepatic circulation is thus interrupted and bile salts are not returned to the liver, the rate of bile acid biosynthesis in the rat is greatly increased (*106*). This may be related to the fact that the rat is exceptional in not possessing a gall bladder. In bile-fistula dogs when the collected bile is fed back to the dog the rate of excretion of bile salt is greatly *increased* (*118*). In the intact dog much of the endogenous bile acid may be transformed by intestinal bacteria to substances that cannot be reabsorbed.

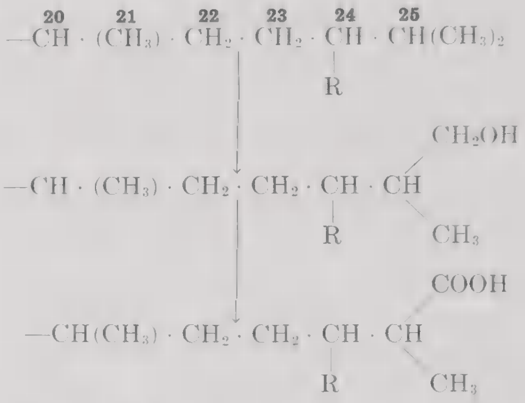
5. *Gallstone Formation*

Gallstones consist in man almost entirely of cholesterol (*119*) and occur very much more commonly in man than in other species. Their formation may possibly be due to failure of man to adapt himself to a relatively high cholesterol (flesh) diet (*107*). Other primates do not have such a diet. Moreover, in man glycine conjugation of bile acids occurs predominantly (*120, 121*) and the glycoconjugates may lack the cholesterol-dissolving power of the tauroconjugates typical of the carnivore (dog) bile (*119*).

6. *Bile Salts and Evolution*

Haslewood (*107*) has suggested that investigation of bile salts may be of value in the elucidation of the evolutionary history of different species. Bile salts (regarded as "primitive") containing C_{27} to C_{29} bile acids have been found in some teleostean and elasmobranch fishes, amphibians, reptiles, and birds, but not in mammals. All animals found so far to possess "primitive" bile salts show other primitive characteristics. Marine invertebrates and other lower animals possess sterols having 28 or 29 carbon atoms, whereas the C_{27} cholesterol is the characteristic sterol of higher animals. One might therefore reasonably expect to find in lower animals C_{28} and C_{29} bile alcohols and acids derived as shown in reaction sequence (1), where R is $\cdot CH_3$ or $\cdot CH_2 \cdot CH_3$.

Such compounds may be unable to undergo oxidation at C-24, which, occurring in the case of cholesterol ($R = H$), leads to the C_{24} bile acids. Hence in an evolutionary sense bile salt formation may be restricted to C_{28} or C_{29} compounds in those animals which possess C_{28} or C_{29} sterols. "Thus a vertebrate having bile salts of this kind may be said to have a



$$\begin{array}{l} \overset{20}{-CH} \cdot \overset{21}{(CH_3)} \cdot \overset{22}{CH_2} \cdot \overset{23}{CH_2} \cdot \overset{24}{\underset{|}{CH}}\!\!\!\!\!\!\underset{R}{} \cdot \overset{25}{CH(CH_3)_2} \\ \downarrow \\ -CH \cdot (CH_3) \cdot CH_2 \cdot CH_2 \cdot \underset{R}{\underset{|}{CH}} \cdot CH\begin{smallmatrix} CH_2OH \\ CH_3 \end{smallmatrix} \\ \downarrow \\ -CH(CH_3) \cdot CH_2 \cdot CH_2 \cdot \underset{R}{\underset{|}{CH}} \cdot CH\begin{smallmatrix} COOH \\ CH_3 \end{smallmatrix} \end{array} \qquad (1)$$

'memory' of a sterol which its ancestors once contained (and which is perhaps still present in the liver as a bile salt precursor). If such a sterol was found, say, in a group of invertebrates, there would be a case for speculation as to whether the line of evolution of the vertebrate could be said to lead back to that invertebrate group" (*107*).

Haslewood also thinks that the finding of "unique" bile salts among animals having "modern" (C_{24}) bile acids, e.g., the 6α-hyocholic and hyodeoxycholic acid of the pig, the phocaecholic acids of seals and walruses, and the pythocholic acid of boas and pythons, may also be of significance in the study of evolutionary history. In such cases, however, it will always be necessary to establish that the "characteristic" bile acid is synthesized in the liver cells of the animal and is not a product of the action of the intestinal flora which may be influenced by the nature of the animal's diet.

7. *Conclusions*

It seems very probable that the bile alcohols and acids are derived from sterol precursors by incompletely understood reactions including inversion of the configurations of the usual 3β-hydroxyl group, further hydroxylations at the 6-, 7-, and 12-positions and oxidation of the C-17 side chain. Whereas sterols are found in the most primitive of organisms, the bile salts make their appearance in animals with calcified skeletons. In higher animals the biochemistry of bile salts is complicated by the occurrence of an enterohepatic circulation and intestinal flora which can effect the hydrolysis of bile salts and transformation of the liberated bile acids. Indeed it is possible that there is no direct formation of deoxycholic acid in animals and that this bile acid is always a product of the action of intestinal flora on cholic acid.

We are still unable to answer the questions raised by Sobotka (*119*):

> "A future comparative biochemistry will be called upon to explain the phylogenetic parallelism and simultaneity of the secretion of bile

> acids with a calcified skeleton, and perhaps connect them with the needs for exogenous antirachitic factors, the development of parathyroid glands, and the beginning of a special cholesterol metabolism based on its endogenous synthesis and correlate these steps in biochemical evolution even with remoter points such as the increased resistance to lead poisoning or susceptibility to certain tumorous growths."

II. The Metabolism of Steroids

A. INTRODUCTION

The complexity of the metabolism of steroids in higher animals is readily appreciated. The steroid hormones and their metabolites alone account for almost two hundred substances isolated from animal tissues and excreta. The diversity of products which may be obtained from a single relatively simple steroid is indicated by the detection of fifty-five distinct substances on incubation of 4-C^{14}-androst-4-ene-3,17-dione with ox adrenal homogenates (*122*). By contrast almost nothing is known of the metabolism of steroids by insects which usually show a nutritional requirement for sterols or by plants and microorganisms which either synthesize these substances or encounter them naturally in their environment. The recently revived interest in the transformation of steroids by microorganisms which may never naturally encounter these substances has indicated the possibility of effecting a wide range of steroid reactions and of preparing many steroids not so far found in animal tissues. These transformations are however not strictly speaking metabolic reactions. Although much has been learned about the occurrence of sterols in invertebrates* nothing is known of their metabolism in these animals.

B. METABOLISM OF STEROLS BY INSECTS

The nutritional requirement of insects for sterols has recently been reviewed (*123*). Free or ester cholesterol is effective in supporting normal growth in many insects and thus it is not surprising that esterase has been reported in the intestines of the wax moth *Galleria mellonella* (*124*) and the American cockroach *Periplaneta americana* (*125*). Evidence was also obtained that the latter insect can degrade the side chain of the cholesterol molecule. The Australian ant *Iridomyrmex detectus* contains cholesterol and is known to produce methylheptenone (*126*). As pointed out by Horning (*123*), this could be readily obtained from the cholesterol side chain at C-17.

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* See Chapter 2 in this volume by W. Bergmann.

It is tempting to speculate that cholesterol is a precursor of hormones required by the insect during the larval stage or during metamorphosis, and this might be thought to gain support from the observation (*127*) that in the larva of the meal worm (*Tenebrio molitor*) the cholesterol requirement decreases with age. However, an alternative explanation of this observation is possible and experimental proof of the transformation of cholesterol into other biologically active substances in insects is lacking.

C. MICROBIOLOGICAL TRANSFORMATION OF STEROIDS

Bile acids, sterols of dietary origin or in the bile, and the metabolites of steroid hormones excreted in the bile are exposed to the action of bacteria in the intestines. The transformations of bile acids by these bacteria have already been referred to (Section I,C,4). Cholesterol is reduced to coprostanol in the intestines (*128*). Estrone and metabolites of progesterone, testosterone (*129*), and hydrocortisone (*130*) have been found in the bile of the ox and of human subjects, but nothing is known of the possible microbiological transformation of these steroids in the intestines. The presence of an enterohepatic circulation of steroids in man was demonstrated by experiments in which C^{14}-labeled estrone was administered intravenously when about 80% of the C^{14} appeared in the urine. When bile was collected from a fistula, 50% of the C^{14} appeared in the bile but in nonfistula subjects only about 7% of the C^{14} was recovered from the feces (*131*). Thus the possibility exists that steroids found in the urine may have been formed by bacteria in the intestines. No systematic study of the steroids in feces has been made, but it has been observed that the androgenic steroids in dried feces of pregnant cows increase in amount on administration of progesterone to the animals (*132*). The following substances have been identified in this material: 5α- and 5β-androstane-3,17-dione, androst-4-ene-3,17-dione, and androst-1,4-diene-3,17-dione (*133*). The occurrence of the last compound as a product of the action of a species of *Fusarium* on progesterone (*134*) suggests that some of these androgens may be formed by microorganisms in the intestines of the cow.

In addition to the intestinal flora, other bacteria and molds have been found to effect transformations of steroids completely foreign to their normal environment (*135*). The enzymes involved are probably induced in the microorganisms, perhaps as a defense against intoxication. By continued exposure of the organism to steroid, high activity of these enzymes may be built up, and microbiological transformation of steroids has become of considerable commercial importance. The reactions catalyzed include hydroxylations, other oxidations and reductions, some of which have not been observed in animal tissues. The discovery that various microorganisms could bring about hydroxylations at various positions in the steroid molecule is of importance from the theoretical as well as the practical point of view since it provides test systems for the study of mechanisms of hydroxylation about which much has still to be learned. As with the mammalian steroid hydroxylating systems, those in microorganisms incorporate oxygen into the hydroxyl group from molecular oxygen but not from water (*136*). A second example of similarity in mechanism of steroid enzymatic hydroxylation, irrespective of the origin of the enzyme system from animal tissues or microorganisms, is provided by the observation that in 11-hydroxylation there is simple replacement of the hydrogen atom in the position to be hydroxylated, and not Walden inversion (*137*).

Animal organisms do not appear to degrade the ring structure of steroids to any significant extent; for example $C^{14}O_2$ could not be detected in the expired air of mice treated with 4-C^{14}-testosterone (*138*). There are, however, numerous examples of the utilization of steroids as the sole source of carbon by microorganisms, and in the case of *Pseudomonas testosteroni* it seems likely that the carbon must be derived from the ring structure of testosterone (*139*).

Other bacteria undoubtedly exist which are able to degrade sterols occurring naturally in their environment. Large amounts of plant and animal sterols continually returned to the soil do not accumulate, and the presence of microorganisms in soil which actively degrade cholesterol has been demonstrated (*140*). Apart from the isolation of a keto acid derived from cholesterol by oxidative fission of the A ring nothing is known of the mechanism of this degradation (*141*).

Although much has been learned about the biosynthesis of sterols in microorganisms, nothing is known of their functions or metabolism in these organisms.

D. ABSORPTION AND METABOLISM OF STEROLS BY ANIMALS

Of all the steroids, only sterols are likely to be present in the diet of animals in significant amounts. It is known that cholesterol is absorbed from the intestines in mammals and birds. Interest in this absorption centers round the fact that feeding cholesterol to certain species of animals results in atherosclerosis. On a body weight basis, small animals such as the rat, guinea pig, and rabbit absorb much more cholesterol than larger animals, including man (*108*). Among the experimental animals studied, the rabbit has an unusually high capacity to absorb cholesterol whereas in man cholesterol absorption is poor even under the most favorable conditions. The absorption of cholesterol is influenced by a number of factors including the presence of other sterols. Thus β-sitosterol is absorbed by rats, rabbits, and man and its presence inhibits cholesterol absorption in chickens, rats, and rabbits (*108*).

As already pointed out (Section I,C,2) the formation of bile acids is the main metabolic pathway of cholesterol in the rat, but this need not necessarily be the case in all species. Comparative studies are lacking.

E. METABOLISM OF STEROID HORMONES

1. *General Aspects of Steroid Hormone Metabolism*

The principal metabolic pathways of steroid hormones observed in man and a limited number of other species are reductive in nature. The Δ^4-3-oxo group is reduced in two stages as shown in Fig. 6a. The 3α-epimer predominates in man. The C-20 oxo group characteristic of the C_{21} compounds may also undergo reduction to 20α- and 20β-hydroxy compounds (Fig. 6b). The reduction of a primary alcoholic group at C-21 to a C-20 methyl steroid (Fig. 6c) is a reaction of minor importance. Metabolic changes which are oxidative in nature include removal of the C-17 side chain by liver enzymes, hydroxylations at 2, 6, 16, 18, and 19 and oxidation of hydroxyl groups, notably those at C-11β and C-17, to oxo groups. Removal of the C-17 side chain may be regarded as a biosynthetic reaction when, occurring in endocrine tissues, it leads to androgenic and estrogenic steroid hormones. Hydroxylations at C-2 and C-19, observed so far only in endocrine tissues, may be biosynthetic reactions leading to aromatization of the A ring, and 18-hydroxylation

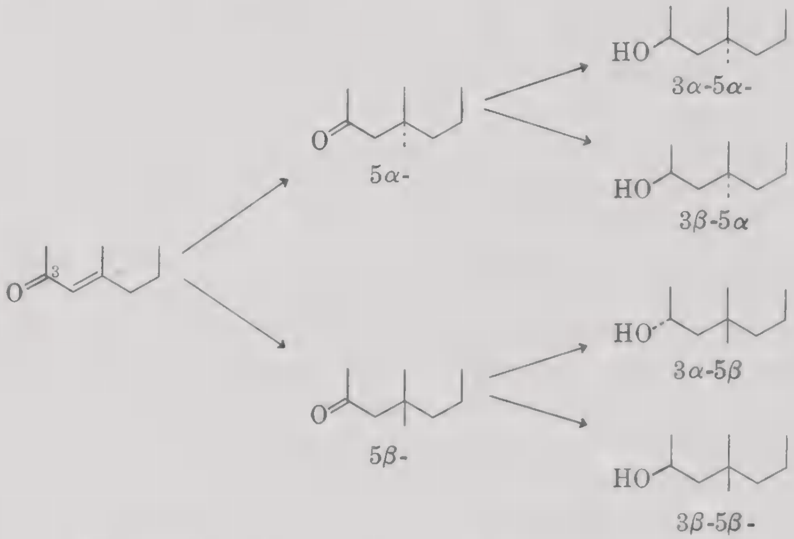

FIG. 6a. Metabolic reduction of the A ring in steroids.

may be a stage in aldosterone biosynthesis. The significance of the 2-O-methylations (2-methoxyestrone (*142*) and 2-methoxyestriol (*143*) have been found in urine in man) is unknown but suggests that 2-hydroxylation may have some role other than biosynthetic. The interesting observation has recently been made that, *in vitro*, adrenals of the non-fatty type, commonly found in ruminants, can introduce a 19-hydroxyl group readily into 11-deoxycorticosterone (DOC). The golden hamster possesses such an adrenal, which in some experiments formed as much 19-hydroxy as 11β-hydroxy compound on incubation with DOC. By contrast the fatty type of adrenals, as found in man, the guinea pig, etc., form 19-hydroxy DOC very slowly if at all (*144*). Hydroxylations at C-6 and C-16 which occur in the liver and possibly other tissues have generally been regarded as catabolic reactions, possibly preceding ring opening, although there is no evidence in support of this. The recent discovery that 3β,16α-dihydroxy-5α-pregnan-20-one, isolated from hog adrenal extracts, causes sodium excretion when tested in the rat suggests a new significance for 16-hydroxylation (*80*).* Hydroxylation at C-6 and C-16 and the oxidations of 11- and 17-hydroxyl groups have been observed in a number of different species, but it should be noted that *good* evidence for the occurrence of 16-hydroxylation of estrogenic steroids (i.e., estriol formation) in species other than man is still lacking.

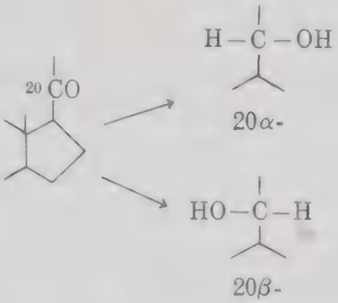

FIG. 6b. Metabolic reduction of the C-20 carbonyl in steroids.

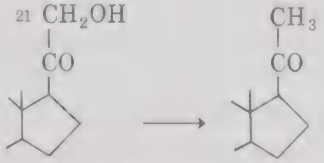

FIG. 6c. Metabolic reduction of the C-21 alcohol group.

It was at one time thought that the 6β-hydroxylations observed with ox endocrine tissue *in vitro* and the isolation of 6α-hydroxy compounds from the urine of human subjects indicated a species difference. However the isolation of a 6α-hydroxy compound on incubation of androstenedione with ox adrenal homogenates (*122*) and the isolation of both 6α- and 6β-hydroxy derivatives of 11β-hydroxyandrostenedione from the urine of guinea pigs (*145*) suggest that these are general reactions.

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* The authors have been unable to confirm this observation.

The general impression that species may differ in their ability to effect the reduction of the 20-oxo group to either the 20α- or 20β-hydroxy group in a wide variety of C_{21} steroids has been supported by recent work. Thus the rat gives predominantly the 20β-epimer *in vivo* (*146*) and *in vitro* (*147*). In man the 20α-epimer is the more usual product *in vivo* (*148*) and *in vitro* (*149*). In this respect the rabbit appears to be similar to man (*150*). The guinea pig however excretes both 20α- and 20β-hydroxy derivatives of hydrocortisone (*145*).

Studies of the metabolism of such C_{21} steroids as progesterone, hydrocortisone, and 11-deoxycorticosterone *in vivo* have been made mostly with human subjects and have indicated that the reduced substances formed are predominantly of the 5β-pregnane series. *In vitro* studies have, however, usually been made with tissues from the rat or the ox, and the reduced steroids formed are predominantly 5α-pregnane derivatives. It is not yet possible to decide if this is due to a species difference or to some other cause. Using a sensitive specific test for 5β-pregnane-3α,20α-diol, this substance was not detected in the urine of rats injected with progesterone (*151*). In a single experiment in which progesterone was incubated with liver from a human subject 5β-pregnane derivatives were predominant among the products (*149*).

In no species is the nature of the estrogenic steroids secreted by the endocrine tissues known. Thus we are unable to say whether the common estrogens, estrone, estradiol-17α, and estradiol-17β are hormones or metabolites. Because it is most active in some biological tests estradiol-17β has been regarded as the ovarian hormone in man. From what is known of the biosynthesis of estrogenic steroids (see p. 181) it would be more reasonable to regard the 17-ketosteroid estrone as the primary substance formed. The interconversion of estrone and estradiol-17β by a variety of tissues from human subjects and animals is well established (*152*) and could account for the appearance of both these substances in the urine. Species differences, however, appear to exist in the stereochemical direction of the reduction of estrone. Thus estradiol-17β but not 17α is found in the urine in man, whereas the urine of pregnant cows (*153*) and goats (*154*) contains the 17α-epimer but no detectable amounts of the 17β-epimer. The formation of the 17α-epimer seems also to be favored in rabbit tissues (*155*). Velle takes the view that in the cow the principal ovarian estrogenic hormone is estradiol-17β and that this is converted to the urinary steroid estradiol-17α outside the ovary (*156*). A satisfactory solution to these problems will be obtained only when reliable methods for the determination of estrogenic steroids in blood have been developed and applied to a comparison of the concentrations of these steroids in blood draining from the estrogen-secreting endocrine glands and in the peripheral circulation.

It has already been pointed out (Section I,B,3,*b*) that there is no satisfactory evidence for the occurrence of estriol in species other than man, and knowledge of the metabolic relationships of the C-16 oxygen-substituted steroids is restricted to man (*157*).

2. *Excretion of Steroid Metabolites*

The products of steroid metabolism have been found in the urine of various species of animals in conjugation with sulfuric and glucuronic acids and possibly other substances. No evidence for differences in mode of conjugation in different species has appeared. Methods for the hydrolysis of conjugates and for the determination of steroids released have been worked out for urines from human subjects, but it is well known that these methods are not always applicable to animal urines, and the quantitative aspects of the study of urinary steroids in animals has suffered accordingly.

Investigations with steroids labeled with radioactive isotopes have drawn attention to the importance of the bile and feces as routes for the excretion of metabolites. In some species these may be the most important routes. For example 65% of the radioactivity of cortisone administered intramuscularly to rats appeared in the feces (*158*) and similar results were obtained with labeled progesterone (*159*) and testosterone (*160*). In the case of human subjects the greater part of the radioactivity appeared in the urine (*159*).

3. *Steroid Hormone Metabolism in Domestic Animals*

The study of steroid hormone metabolism in domestic animals is of obvious importance in animal husbandry, and as in the case of man the investigation of urinary metabolites can provide much valuable information. However, whereas urinary steroids have been thoroughly investigated in man and much emphasis has been placed on the development of *reliable* methods for the measurement of these steroids, comparatively little work has been done along the same lines with domestic animals.

From a review of existing knowledge (*162*) it would appear that different species show quite different excretory patterns (Table IV). The

TABLE IV
URINARY EXCRETION OF STEROIDS BY ANIMALS

A. URINARY ESTROGENS

Animal	Estrone	Estradiol-17β	Estradiol-17α	Estriol	Equilin	Equilenin
Cow (pregnant)	+		+			
Bull	+					
Goat (pregnant)	+		+			
Sow (pregnant)	+					
Boar	+	+				
Mare (pregnant)	+	+	+		+	+
Stallion	+	+				
Sheep (pregnant)	+	+				
Woman (pregnant)	+	+		+		

B. URINARY METABOLITES OF PROGESTERONE (-DIOLS)

Animal	5α-Pregnane		5β-Pregnane-	
	-3α,20α-diol	-3β,20α-diol	-3β,20β-diol	-3α,20α-diol
Cow (pregnant)	+	+		+
Bull				+
Goat (pregnant)				+
Mare (pregnant)	+	+	+	+
Sheep (pregnant)				+
Woman (pregnant)	+			+
Man				+

C. URINARY C_{19} STEROIDS

Animal	5α-Androstane			5β-Androstane		3α-Hydroxy-5β-androstan-17-one	3β-Hydroxy-androst-5-en-17-one	3α-Hydroxy-5α-androstan-17-one	3β-Hydroxy-5α-androstan-17-one	3α-Hydroxy-5β-androstane 11,17-dione
	-3β,17α-diol	-3α,16α-diol	-3β,16β-diol	-3α,17α-diol	-3α,17β-diol					
Cow	+			+		+		+	+	+
Goat	+			+		+	+	+		+
Mare		+	+				+		+	
Man					+	+	+	+	+	+

pregnant mare shows a curious change in urinary excretion of estrogens during pregnancy. Estrone and estradiol-17β are predominant up to about mid-pregnancy. In the later stages estradiol-17β is progressively replaced by equilin to equilenin. It is not known whether this is a true metabolic change or a change in the method of excretion of these steroids. The total amount of estrogenic material, as judged by bioassay, also falls after mid-pregnancy. The cow in contrast with the mare excretes estrogenic material in the urine at a steadily increasing rate as pregnancy advances. The significance of these changes would be easier to interpret if the actual substances concerned were determined by reliable chemical methods.

One of the remarkable features of the cattle urines is the low concentration of neutral steroids. Thus the major neutral steroid of pregnant cow's urine is 5β-androstane-3α,17α-diol present in amounts of about 0.2 mg. per liter. From 20 to 40 mg. "apparent ketosteroid" were found per liter of cattle urine, but of this only about 0.3 mg. per liter is true ketosteroid (*159*). The high values have been attributed to interference from ionone compounds in the diet. Of the species investigated so far, appreciable amounts of ketosteroids have been found only in the urine of man and guinea pigs (*160–164*).

F. CONCLUSIONS

The principal known pathways of cholesterol metabolism in higher animals are the formation of steroid hormones and of bile acids. The latter is probably quantitatively the more important. Nothing is known of sterol metabolism in lower animals or in plants or microorganisms. The principal known metabolic transformations of steroid hormones are reductions, oxidations, and conjugations. The last results in the formation of more water-soluble substances which appear in the urine. There are suggestions that fecal excretion of steroids may be of more importance in some species than others.

It is unfortunate that knowledge of steroid metabolism is restricted almost exclusively to higher animals, and despite numerous recorded species differences no body of facts exists which could be called a comparative biochemistry of steroid metabolism. This situation has almost certainly been brought about by the influence of medical thinking and the demands for the solution of the problems of endocrine diseases in man. It is particularly striking that after the unprecedented expenditure of time and effort on the production and study of cortisone and hydrocortisone it is now realized that these substances cure no disease and that their prolonged administration for the relief of symptoms is accompanied by the risk of unpleasant and even dangerous side effects.

It is not unreasonable to suppose that a broad basis of knowledge such as might have been obtained from a study of the comparative biochemistry of the adrenocortical hormones would have been of inestimable value here. As Albert has pointed out, mammals did not invent the hormones (*165*), and these substances must have been subjected to evolutionary experiments. Modern techniques are capable of revealing the results of such experiments, and it would be surprising if these results did not do much to clarify problems in mammalian steroid biochemistry. Moreover, it would be extremely interesting to know which was the first steroid hormone. The requirement of certain lower organisms for cholesterol as a growth factor may prove to be a starting point for such investigations.

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CHAPTER 4

Bile Salts: Structure, Distribution, and Possible Biological Significance as a Species Character

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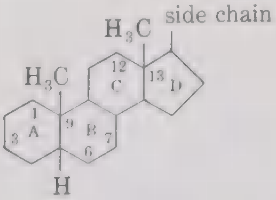
I. Structure

A. INTRODUCTION

The molecules of all bile salts are of the "detergent" type: that is to say, they possess a hydrocarbon, fat-dissolving part and a polar, water-attracting portion. The "fatty" part consists of the bulk of the steroid nucleus, including the methyl groups at C-9 and C-13 (I). A *cis* junction between rings A and B ensures that the nucleus is so folded that it forms what in a Stuart molecular model looks like a sort of "fist" (*1*), on the flatter side of which are arranged the polar α-orientated nuclear hydroxyl groups which, together with the —COOH, —COO$^-$, or —SO_3^- groups at the end of the side chain, can easily lie



(I)

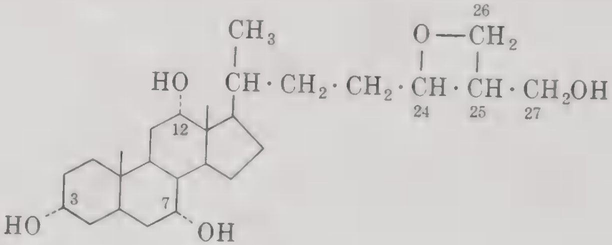
together in a single plane at a water–fat interface. The hydroxyl groups are often so distributed that hydration can readily occur, and this will assist dispersion in the watery phase. The remainder of the molecule will dissolve in the fatty phase, and so emulsification can easily be brought about. A consequence of the molecular shape is that separate nuclei will adhere to each other by van der Waals forces, and thus clumping to form micelles occurs at all except very great dilutions. "Critical micellar concentrations" have in fact been measured for certain bile salts (*2*).

Bile salts are substances, derived from sterols available to animal organisms, which have been perfected during evolution to assist in the emulsification, hydrolysis, and absorption of fats. At least four distinct chemical types fulfilling these conditions have been found in nature and are described below.

B. Types of Bile Salts

1. C_{27} (*or* C_{28}) *Alcohols*

The longest known substance in this group is scymnol, discovered by Hammarsten in 1898 (*3*). It has been isolated after alkaline hydrolysis of all elasmobranch bile examined, but has not yet been obtained from any other source. The formula (II) has been fully verified (*4, 4a*).



(II)

Scymnol itself does not, apparently, occur in the bile. The "native" sulfate is that of a hexahydric alcohol; the sulfate group is on C-27, or, less probably, on C-24, and the oxide ring results when this group is removed by treatment with alkali (*5*), as with ranol sulfate from frog bile (*6*). In view of this, scymnol has been renamed "anhydroscymnol" and the bile salt is "scymnol sulfate" (*4a*, *5*). True "scymnol"

$$\text{side-chain:—}CH(CH_3)\cdot CH_2\cdot CH_2\cdot \overset{24}{CHOH}\cdot CH(CH_2OH)_2$$

has now been obtained from shark bile and also by partial synthesis (*5a*).

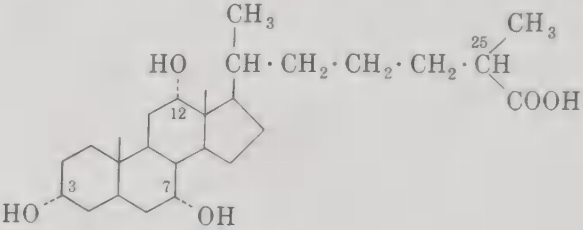
Pentahydroxybufostane (perhaps $C_{28}H_{50}O_5$) was isolated from toad bile by Kazuno (*7*), who seems to have satisfactorily established that, like scymnol, it contains the cholic acid nucleus. The remaining details of the formula await final elucidation. It will be especially interesting if it can be conclusively shown that the chief biliary alcohol, pentahydroxybufostane, does, as Kazuno claims, contain 28 carbon atoms in its molecule, for this will imply that the molecules of a sterol occurring in major amounts in toad liver must have at least as many. Other alcohols called tetrahydroxynorbufostane, tetrahydroxycholane, and isotrihydroxycholene ($C_{24}H_{40}O_3$) have been reported from toad bile (7, 8). Tetrahydroxyisohomocholane, (?) $C_{25}H_{44}O_4$, was obtained by Okasaki (9) from the bile of the newt *Diemyctylus pyrrhogaster.* The frog *Rana temporaria* has, as its chief bile salt, ranol sulfate; the parent alcohol, ranol, can be easily obtained as a crystalline tetraacetate, probably $C_{26}H_{42}CHOH \cdot (OCOCH_3)_4$ (5). Ranol does not contain the simple cholic acid nucleus and probably has the same nuclear structure as cyprinol, a very similar alcohol from Cyprinidae (*10, 11*). The frog *Rana catesbiana,* in addition to ranol, is said (*12, 13*) to have a biliary trihydroxyhomocholane, $C_{25}H_{44}O_3$. Biliary alcohols of unknown constitution occur in a coelacanth (*14*).

The very primitive chordate, hagfish (Class Marsipobranchii), has been shown to contain the disulfate of an alcohol (myxinol) which does not have the cholic acid nucleus. This alcohol sulfate, which was the chief or only bile salt in the specimens examined, has been isolated from the Atlantic species *Myxine glutinosa* as well as the Pacific form, *Eptatretus stoutii* (*15*).

2. *C_{27} (or C_{28}) Acids*

The isomeric acids, trihydroxybufosterocholenic and trihydroxyisosterocholenic, from toad bile, were described by T. Shimizu and his colleagues (*16*); they have both been degraded to bisnorcholic acid (*16, 16a*), and hence presumably contain the cholic acid nucleus and side chain as far as C-22 (II). A double bond between C-22 and C-23 follows, and the complete formula is given as $C_{28}H_{46}O_5$, based on a "sterocholanic acid," $C_{28}H_{48}O_2$. Claims to have prepared naturally occurring 24-methyl coprostanic acids from cholanic acids (*17, 18*) have been made. If C_{28} acids do in fact constitute an important part of toad bile salts, then their occurrence raises the same interesting questions as appear if pentahydroxybufostane is a C_{28} alcohol (see above).

Tetrahydroxysterocholanic and tetrahydroxyisosterocholanic acids have been isolated from turtle and tortoise bile (*19–21*) and formulated as $C_{27-28}H_{46-48}O_6$. Each forms a lactone, and the former contains the cholic acid nucleus (*22*). Acids originally named α- and β-trihydroxybisnorsterocholanic, $C_{26}H_{44}O_5$, and found in the frog *Rana catesbiana* (*23, 24*) were later shown to have the formula $C_{27}H_{46}O_5$ and to be C-25 epimers of $3\alpha,7\alpha,12\alpha$-trihydroxycoprostanic acid (III) (*25*). One of these acids, 25α (*4*), was found in the biles of crocodiles and an alligator (*26*), and converted to its "stem acid," 25α-coprostanic acid, $C_{27}H_{46}O_2$ (*27*). Both 3α,7α,12α-trihydroxycoprostanic acids were obtained from the frog *Rana nigromaculata* (*28*). Δ^{23}-3α,7α,12α-Trihydroxycoprostenic acid, from the toad *Bufo vulgaris japonicus,* has been hydrogenated to give 3α,7α,12α-trihydroxy-25α-coprostanic acid (*29*).

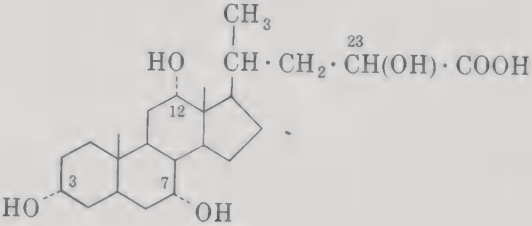


(III)

The bile of the monitor *Varanus niloticus* yielded (*30*) varanic acid, $C_{27}H_{46}O_6$, of unknown constitution. There is little doubt that more hydroxylated coprostanic acids remain to be discovered. "Tetrahydroxynorsterocholanic acid" (*31*) formerly written as $C_{27}H_{46}O_6$ is not now believed to have a C_{27} formula; it is discussed below.

3. C_{24} *Acids*

Most of these are derivatives of cholanic acid, $C_{24}H_{40}O_2$. The only known natural tetrahydroxycholanic acid is 3α,7α,12α,23-tetrahydroxycholanic (IV); it has recently (*32, 33*) been identified in the bile of Pinnepedia and of two species of snakes of the genus *Bitis.*



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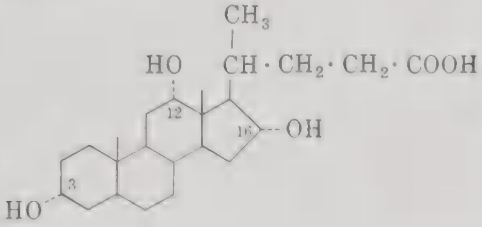
Phocaecholic acid is 3α,7α,23-trihydroxycholanic (*33, 34*), and the recently discovered bitocholic acid (*33*) is 3α,12α,23-trihydroxycholanic. In none of these 23-hydroxylated cholanic acids is the optical configuration at C-23 known; it seems possible that acids having both configurations at C-23 may exist together in biles.

Snakes of the Family Boidae were found (*35, 36*) to have pythocholic (3α,12α,16α-trihydroxycholanic) acid (V). The very common cholic acid is 3α,7α,12α-trihydroxycholanic, whose allo(5α)epimer also occurs (*37*).

Hyocholic acid (VI) from pig bile (*37a*) is 3α,6α,7α-trihydroxycholanic (*38–40*).



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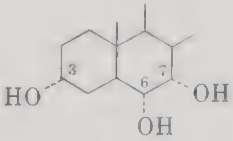

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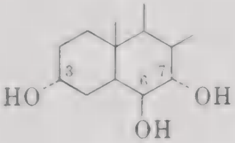
Two acids recently found in rat bile are 3α,6β,7α- and 3α,6β,7β-trihydroxycholanic acids (α- and β-muricholic acids, VII, VIII) (*41*), and



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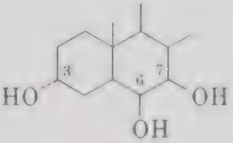
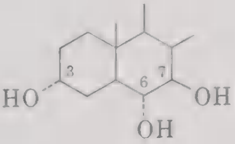


(VIII)

3α,6α,7β-trihydroxycholanic acid (ω-muricholic acid, IX) is formed (*41*, *42*) when rats are treated with 3α,6α-dihydroxycholanic acid (hyodeoxycholic acid) well known as a constituent of pig bile (see *38*). 3β,6α- and 3α,6β-Dihydroxycholanic acids have also been isolated from pig bile (*30*, *42–44*), as well as the keto acid, 3α-hydroxy-7-oxoallo(5α)cholanic (X) (*45*), which has a *trans* A/B junction and is



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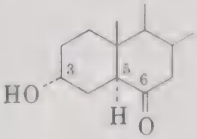
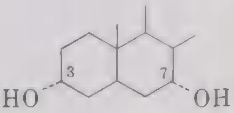


(X)

probably formed from the corresponding cholanic (5β) acid during alkaline hydrolysis of the bile. Another widely distributed acid is chenodeoxycholic (3α,7α-dihydroxycholanic) (XI). Its epimer, 3α,7β-dihydroxycholanic, ursodeoxycholic acid (XII) has been found in coypu



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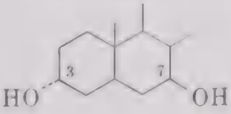
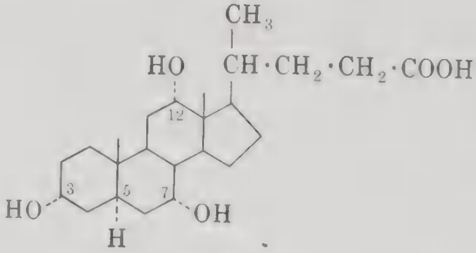


(XII)

(*46*) and rat (*47*) bile as well as in men (*48*) and bears. Recently, 3α,7β,12α-trihydroxycholanic acid has been found to be formed in rat bile in metabolic experiments (*48*). Coypu and guinea pig bile also contain 3α-hydroxy-7-oxocholanic acid (*46, 49*); other keto acids have been isolated from various biles (*4*).

The well-known deoxycholic acid, 3α,12α-dihydroxycholanic acid, is probably (*48*), like some of the acids mentioned above, and also lithocholic (3α-hydroxycholanic) acid, made from other bile acids by microbial action in the intestine rather than directly by the liver itself.

An acid which was given the formula $C_{27}H_{46}O_6$ and named "tetrahydroxynorsterocholanic acid" was isolated by Ohta in 1939 (*31*) from the bile of the Japanese "Gigi" fish, *Pelteobagrus nudiceps.* The same acid was later obtained from other fishes, and Yamasaki (*50*) found it in chicken bile. Ohta suggested that it was a 3,6,12,24-tetrahydroxycoprostanic acid, and Isaka (*51*) claimed to support this structure by conversion of Ohta's acid to hyodeoxycholic and to 12-oxocholanic acids. On infrared spectral evidence, the writer and Wootton (*52*) suggested that Ohta's acid must have hydroxyl groups at 3α, 7α and 12α in a cholane structure. When Anderson *et al.* were able to examine a larger quantity of the ethyl ester of this acid, isolated from King penguin bile (*53*), they concluded that the ester might well be isomeric with ethyl cholate. Recently, Ohta's acid has been proved to be allo(5α)cholic acid, i.e., 3α,7α,12α-trihydroxyallo(5α)cholanic acid (XIII) (*37*).



(XIII)

4. *Nature of the Conjugates*

With possible exceptions in some amphibian bile (*7*), all bile acids and alcohols are believed to occur, at least in healthy animals, in conjugated forms. Alcohols are conjugated with sulfate and the bile salts must, as a result of the dissociation of the $—SO_3H$ group, occur at all hydrogen ion concentrations likely to be compatible with life as the ions $R·O·SO_3^-$. It is generally assumed, though not yet proved in any single case, that sulfate conjugation occurs at a primary alcohol group at the end of the side chain, giving sulfates of the type $R·CH_2·O·SO_3^-$.

The resulting bile salt is similar to the taurine ($NH_2 \cdot CH_2 \cdot CH_2 \cdot SO_3H$) conjugates which occur with all bile acids ($R \cdot COOH$) and have the general formula $R \cdot CO \cdot NH \cdot CH_2 \cdot CH_2 \cdot SO_3^-$. In some mammals there are also glycine ($NH_2 \cdot CH_2 \cdot COOH$) conjugates of bile acids ($R \cdot COOH$), and these must exist partly as $R \cdot CO \cdot NH \cdot CH_2 \cdot COOH$ molecules and partly as the ions $R \cdot CO \cdot NH \cdot CH_2 \cdot COO^-$. The precise distribution of glycine conjugation is not known; it may possibly be confined to the Eutheria. A few vegetarian species (e.g., rabbit, pig, coypu) have a very high proportion of their bile acids in glycine conjugation, although taurine conjugates do occur in these animals (see, e.g., reference *54*). In the human infant, glycine conjugation is only gradually established (*48*).

II. Distribution

The distribution of the different types of bile salts referred to above is shown in Table I. In this table, findings are given as stated in the original literature. Where the present writer or the author of the work regards the findings as doubtful, this is indicated by a query (?). Nomenclature of species is shown, in general, as it appears in the original publications.

III. Bile Salts as a Species Character

A. GENERAL

The information in Table I may be taken as substantially correct, and it shows that there are definite species variations in the chemical nature of bile salts. Apart from the evolutionary aspect, discussed below, one may notice that closely related species have similar bile salts and that these are sometimes confined to a single group. For example, all the Cyprinidae examined have cyprinol, two of the three investigated species of Ranidae have ranol, all the Boidae have pythocholic acid, the only two snakes examined of the genus *Bitis* have bitocholic acid, and only Suidae have hyocholic acid and its relatives. In larger groups, the Selachii have scymnol, Chelonia have tetrahydroxysterocholanic acid, and all the Pinnipedia, phocaecholic acid. On the other hand, distribution of some substances (for example, the trihydroxycoprostanic acids, Ohta's acid, deoxycholic acid, ursodeoxycholic acid and the keto acids) is "patchy" and apparently not governed by evolutionary relationships; perhaps the same remarks should apply to glycine conjugation. Thus, it is clear that if bile salts are to be used as a species character, care will have to be taken in selecting substances for this purpose. Valuable

TABLE I

Natural Distribution of Bile Salts[a]

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
Invertebrates			
Crayfish and crabs	Bile salts detected, but not identified, in digestive juices		*(55)*
Vertebrates			
MARSIPOBRANCHII			
MYXINIDAE			
Hagfish, *Myxine glutinosa* and *Eptatretus stoutii*	Myxinol	Sulfate	*(15)*
ACTINISTIA			
Coelacanth, *Latimeria chalumnae*, Smith	Alcohol(s) of unknown chemical constitution	Sulfate	*(14)*
SELACHII			
CARCHARINIDAE			
Galeocerdo arcticus	Anhydroscymnol	Sulfate	*(56)*
SQUALIDAE			
Squalus acanthias	Anhydroscymnol	Sulfate	*(57)*
		Not glycine	*(58)*
Squalus sucklii	Anhydroscymnol	Sulfate	*(59)*
Scymnus borealis (*Somniosus microcephalus*, Schn.)	Anhydroscymnol; scymnol	Sulfate	*(3,5a)*
ISURIDAE			
Isurus glaucus	Anhydroscymnol	Sulfate	*(60)*
? Two species of shark	Anhydroscymnol; cholic acid; a ?C_{27} acid	—	*(61)*
RAIIDAE			
Raia batis	Anhydroscymnol	Sulfate	*(57)*
TRYGONIDAE			
Dasyatis akajei	Anhydroscymnol; cholic acid		*(62)*
PISCES			
ACIPENSERIDAE			
Acipenser (Huso) huso *A. stellatus* *A. güldenstädti*	Cholic acid; allocholic acid	Taurine	*(63)*
CLUPEIDAE			
Engraulis japonicus	Cholic acid; chenodeoxycholic acid	Taurine	*(31)*

TABLE I (*Continued*)

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
SALMONIDAE			
Salmo (*Oncorhyncus*) *milktschitsch*	Cholic acid	Taurine	(*31*)
Salmo trutta and *Salmo irideus*	—	Not glycine	(*58*)
ESOCIDAE			
Esox lucius	Cholic acid	Taurine	(*64*)
PLECOGLOSSIDAE			
Plecoglossus altivelis	Cholic acid	Taurine	(*31*)
CYPRINIDAE			
Cyprinus carpio	(?) Cholic acid Cyprinol	Sulfate	(*10*, *11*, *31*)
Tinca vulgaris			
Leuciscus cephalus			
Leuciscus rutilus			
Carassius auratus	(?) Cholic acid	? Taurine	(*31*)
SILURIDAE			
Silurus glanis	Cholic acid	—	(*64*)
"Gigi" fish, *Pelteobagrus nudiceps* (Sauvage)	Cholic acid; "tetrahydroxynorsterocholanic" acid[b]	Taurine	(*31*)
Parasilurus asotus	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)
COBITIDAE			
Misgurnus anguillicaudatus	Alcohol, $C_{30}H_{54}O_6$	Sulfate	(*65*)
ANGUILLIDAE			
Eel (Japanese)	Cholic acid	Taurine	(*31*)
CONGRIDAE			
Conger myriaster	Cholic acid	Taurine	(*66*)
Anago anago	Cholic acid	Taurine	(*31*)
MURAENIDAE			
Muraenesox cinereus	Cholic acid	Taurine	(*31*)
BELONIDAE			
Belone vulgaris	Cholic acid	Taurine	(*67*)
MUGILIDAE			
Mugil cephalus	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)
GADIDAE			
Gadus morrhua	Cholic acid; ?deoxycholic acid	Taurine	(*30*, *63*, *68*)
Gadus macrocephalus	Cholic acid	Taurine	(*31*)
Theragra chalcogramma	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)
EPINEPHELIDAE			
Stereolepis ischinagi	Cholic acid	Taurine	(*31*)
SERRANIDAE			
Morone labrax	—	Not glycine	(*58*)

TABLE I (*Continued*)

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
PERCIDAE			
Perca fluviatilis	—	Taurine	(*63*)
SPARIDAE			
Pagrosomus major	Cholic acid	Taurine	(*31*)
Sparus (Milio) macrocephalus	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)
SIGANIDAE			
Siganus fuscescens	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)
SCIAENIDAE			
Sciaena (Nibea) mitsukurii	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)
SCOMBRIDAE			
Thunnus thynnus	Cholic acid	Taurine	(*31*, *69*)
Scomberomorus niphonius	Cholic acid	Taurine	(*31*)
Euthynnus (Katsuwonus) pelamis	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)
XIPHIIDAE			
Xiphias gladius	Cholic acid	Taurine	(*31*)
CARANGIDAE			
Seriola quinqueradiata	Cholic acid; steroid ?$C_{24}H_{42}O_5$	Taurine	(*31*, *70*)
BOTHIDAE			
Rhombus (Scophthalmus) maximus	Cholic acid	Taurine	(*63*)
Paralicthys olivaceus	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)
PLEURONECTIDAE			
Pleuronectes platessa	Cholic acid	Taurine; not glycine	(*30*, *58*)
Pleuronectes flesus	—	Not glycine	(*58*)
GOBIIDAE			
Periophthalamus cantonensis *Acanthogobius flavimanus*	Cholic acid; chenodeoxycholic acid	—	(*71*)
SCORPAENIDAE			
Sebastodes matsubarae *Sebastodes inermis*	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)
Inimicus japonicus	Cholic acid; chenodeoxycholic acid; "tetrahydroxynorsterocholanic" acid[b]	—	(*72*)
BALISTIDAE			
Monocanthus (Stephanolepis) cirrhifer	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)

TABLE I (*Continued*)

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
TETRODONTIDAE			
Tetrodon porphyleus	Cholic acid; "tetrahydroxynorsterocholanic" acid^b	Taurine	(*51, 73*)
FAMILIES UNTRACED			
"Bari"	Cholic acid; chenodeoxycholic acid; "tetrahydroxynorsterocholanic" acid^b	—	(*73*)
"Beisuke"	Cholic acid; chenodeoxycholic acid	Taurine	(*31*)
AMPHIBIA			
SALAMANDRIDAE			
Newt, *Diemyctylus pyrrhogaster*	Cholic acid; deoxycholic acid; alcohol ?$C_{25}H_{44}O_4$; other alcohols	Sulfate	(*9*)
RANIDAE			
Rana catesbiana	"Tetrahydroxycholane" (? = ranol), etc.	Sulfate	(*12, 13*)
	"trihydroxycholenic acid"; $3\alpha,7\alpha,12\alpha$-trihydroxy-25α- and 25β-coprostanic acids	Taurine	(*23, 24*)
Rana temporaria	Ranol	Sulfate	(*5*)
		No glycine	(*58*)
Rana nigromaculata	$3\alpha,7\alpha,12\alpha$-trihydroxy-25α and -25β-coprostanic acids	Taurine	(*28, 65*)
BUFONIDAE			
Toad, *Bufo vulgaris japonica*	"Bufodeoxycholic acid"	Taurine	(*74*)
	Pentahydroxybufostane; tetrahydroxynorbufostane; tetrahydroxycholane	Sulfate	(*7, 75*)
	Trihydroxybufosterocholenic acid; trihydroxyisosterocholenic acid	—	(*16, 16a*)
	Δ^{23}-$3\alpha,7\alpha,12\alpha$-Trihydroxy-25α-coprostenic acid	—	(*29*)
	Isotrihydroxycholene		(*8*)

TABLE I (*Continued*)

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
REPTILIA			
CROCODYLIDAE			
Alligator mississippiensis	$3\alpha,7\alpha,12\alpha$-Trihydroxy-25α-coprostanic acid	Taurine	(*26*)
Caiman crocodilus			
Crocodylus niloticus			
Crocodylus johnsonii	—	Taurine	(*58*)
CHELONIA			
TESTUDINIDAE			
Emys orbicularis	Tetrahydroxysterocholanic acid (lactone)	—	(*20*)
CHELONIDAE			
Amyda japonica	Tetrahydroxysterocholanic acid; tetrahydroxyisosterocholanic acid; heterocholic acid	—	(*19, 21, 22*)
Chelone midas	2 Unidentified acids	Taurine	(*30, 58*)
SQUAMATA			
VARANIDAE			
Varanus niloticus	Varanic acid	—	(*30*)
OPHIDIA (SERPENTES)			
BOIDAE			
Constrictor constrictor	Pythocholic acid	Taurine	(*35, 48*)
Constrictor occidentalis	Pythocholic acid; cholic acid	—	(*35*)
Constrictor imperator	Pythocholic acid	—	(*35*)
Python reticulatus	Pythocholic acid; cholic acid; 3,12-dihydroxy-7-oxocholanic acid	Taurine	(*36, 58, 76*)
Python sebae	Pythocholic acid	—	(*35*)
Python molurus	Pythocholic acid	Taurine	(*35*)
Python tigris	—	Taurine	(*77*)
Python spilotus	—	Taurine	(*58*)
Boa canina	Pythocholic acid; cholic acid	—	(*35*)
Eunicotes murinus	Pythocholic acid; cholic acid	—	(*36*)
Epicrates cenchris	Pythocholic acid	—	(*35*)
COLUBRIDAE			
Elaphe quadrivirgata	Cholic acid	Taurine	(*77*)
Elaphe carinata	Cholic acid	—	(*78*)

TABLE I (*Continued*)

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
Boiga dendrophilus	Cholic acid	—	(*30*)
Drymarchon courais couperi	Cholic acid	—	(*30*)
ELAPIDAE			
Bungarus multitinctus	Cholic acid	—	(*78*)
Dendraspis viridis	Cholic acid	—	(*30*)
Naja nivea	Cholic acid	—	(*30*)
VIPERIDAE			
Bitis gabonica *Bitis arietans*	(Probably) cholic acid; bitocholic acid; $3\alpha,7\alpha,12\alpha,23$-tetrahydroxycholanic acid; allocholic acid	Taurine	(*30*, *33*, *58*)
CROTALINAE			
Crotalus terrificus	Cholic acid	—	(*77*, *79*)
Crotalus horridus	Cholic acid	—	(*30*)
Crotalus oreganus	Cholic acid	—	(*30*)
Crotalus adamanteus	—	Taurine	(*58*)
AVES			
CORVIDAE			
Crow, *Corvus coronoides japonensis*	Cholic acid; chenodeoxycholic acid	—	(*80*)
Magpie, *Pica pica serica*	Cholic acid; chenodeoxycholic acid	—	(*80*)
PHASIANIDAE			
Domestic chicken	Chenodeoxycholic acid	—	See (*53*)
	Isolithocholic acid	—	(*81*)
	Cholic acid; "tetrahydroxynorsterocholanic" acid[b]	—	(*50*)
	3α-Hydroxy-7-oxocholanic acid; 3-oxochola-4,6-dienic acid[c]		(*82*, *83*)
		Taurine, not glycine	(*58*)
Phasianus colchicus karpowi	Chenodeoxycholic acid	—	(*84*)
Meleagris gallopavo	Cholic acid; chenodeoxycholic acid	—	(*85*)
ANATIDAE			
Domestic duck	Cholic acid; chenodeoxycholic acid	—	(*85*)
Anas penelope	Cholic acid; chenodeoxycholic acid	—	(*85*)
Domestic goose	Chenodeoxycholic acid	—	See (*53*)

TABLE I (*Continued*)

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
STRIGIDAE			
Owl	Cholic acid; chenodeoxycholic acid	—	(*86*)
Bubo bubo		Not glycine	(*58*)
SPHENISCIDAE			
King penguin, *Aptenodites patagonica*	Cholic acid; chenodeoxycholic acid; "tetrahydroxynorsterocholanic" acid^b	—	(*53*)
		Not glycine	(*58*)
FALCONIDAE			
Kite, *Milvus lineatus lineatus*	Chenodeoxycholic acid; (probably) Ohta's acid^b	—	(*80*)
Aquila chrysaetos, Terathopius ecaudatus	—	Not glycine	(*58*)
BALAENICIPITIDAE			
Balaeniceps rex	—	Not glycine	(*58*)
CASUARIIDAE			
Casuarius rogersi	—	Not glycine	(*58*)
BUCEROTIDAE			
Bycanistes cylindricus	—	Not glycine	(*58*)
MAMMALIA			
MARSUPALIA			
PHALANGERIDAE			
Koala, *Phascolarctos cinereus*	3α-Hydroxy-7-oxocholanic acid	Taurine	(*11, 64*)
MACROPODIDAE			
Kangaroo, *Macropus giganteus*	Cholic acid; deoxycholic acid; chenodeoxycholic acid	Glycine(?); taurine	(*77, 87*)
Macropus ruficollis	Cholic acid	Probably not glycine	(*30, 58*)
DIDELPHIDAE			
Opossum, *Didelphis marsupialis virginiana*	Cholic acid; chenodeoxycholic acid	Taurine; no glycine; no free acid	(*88*)
EUTHERIA			
ERINACIDAE			
Hedgehog, *Erinaceus europaeus*	Cholic acid	—	(*30*)
CEBIDAE			
Capuchin, *Cebus fatuellis*	Cholic acid; deoxycholic acid	Taurine	(*89*)

TABLE I (*Continued*)

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
"*Pithecus cyclopis*"	Cholic acid; deoxycholic acid; chenodeoxycholic acid	Glycine	(*90*)
CERCOPITHECIDAE			
Cercopithecus aethiops tantulus *Cercopithecus nictitans martinii*	Cholic acid; deoxycholic acid; chenodeoxycholic acid; $3\alpha,12\alpha$-dihydroxy-7-oxocholanic acid	Taurine; glycine	(*82*)
Cercopithecus mona	Cholic acid; deoxycholic acid; chenodeoxycholic acid; lithocholic acid	Taurine; glycine	(*82*)
Olive baboon, *Papio anubis*	Cholic acid	—	(*30*)
Rhesus, *Macacus mulatatto*	Cholic acid	—	(*30*)
Mandrillus leucophaeus	Cholic acid	—	(*30*)
PONGIDAE			
Chimpanzee, *Pan satyrus*	Cholic acid; deoxycholic acid; chenodeoxycholic acid	Taurine; glycine	(*30, 82*)
HOMINIDAE			
Man	Cholic acid; deoxycholic acid; chenodeoxycholic acid; ursodeoxycholic acid; lithocholic acid	Taurine; glycine	(*48, 77, 82*)
LEPORIDAE			
Domestic rabbit	Cholic acid; deoxycholic acid; lagodeoxycholic acid;[d] lithocholic acid	Glycine	(*48, 91*)
Hare, *Lepus timidus*	Deoxycholic acid	—	(*34*)
SCIURIDAE			
Citellus mongolicus ramosus	Cholic acid	Taurine	(*50*)
MURIDAE			
Laboratory rat	Cholic acid; chenodeoxycholic acid; ursodeoxycholic acid; α- and β-muricholic acids	Taurine; glycine	(*41, 47, 48*)
Laboratory mouse	Cholic acid; α- and β-muricholic acids		(*30, 41, 48*)
CAVIIDAE			
Domestic guinea pig	Chenodeoxycholic acid; 3α-hydroxy-7-oxocholanic acid	Glycine; taurine	(*48, 49*)
	Cholic acid and the above acids (fistula bile)	Taurine	(*92a*)

TABLE I (*Continued*)

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
CAPROMYIDAE			
Coypu, *Myocastor coypus*	Cholic acid; chenodeoxycholic acid; ursodeoxycholic acid; 3α-hydroxy-7-oxocholanic acid	Glycine; taurine	(*46, 58, 93*)
THRYONOMYIDAE			
Thryonomys swinderianus	Chenodeoxycholic acid; deoxycholic acid; (probably) ursodeoxycholic acid	Taurine	(*94*)
BALAENOPTERIDAE			
Fin whale, *Balaenoptera physalus*	Cholic acid; deoxycholic acid	Taurine; glycine	(*30, 95*)
Balaenoptera sibbaldi	Cholic acid	—	(*30*)
Balaenoptera borealis	Cholic acid	Taurine	(*95*)
CANIDAE			
Dog	Cholic acid; chenodeoxycholic acid; deoxycholic acid	Taurine	(*77, 86, 96, 97*)
Dhole, *Canis dukhunensis*	Cholic acid	—	(*30*)
Wolf, *Canis lupus*	—	Taurine	(*77*)
Fox, *Vulpes pecculiosus* (Jap.)	Cholic acid; deoxycholic acid	Glycine	(*98, 99*)
Nyctereutes viverrinus (*procyonides*)	Cholic acid; deoxycholic acid	—	(*100*)
URSIDAE			
Ursus arctos isabellinus	? Deoxycholic acid	Glycine	(*101*)
Ursus thibetanus japonicus	Chenodeoxycholic acid; ursodeoxycholic acid	—	(*102*)
"Japanese bear"	Cholic acid; chenodeoxycholic acid; ursodeoxycholic acid	Taurine; glycine	(*103, 104*)
Ursus americanus	Cholic acid	—	(*30*)
Helarctos malayanus	Cholic acid	—	(*30*)
Polar bear, *Thalarctos maritimus*	Cholic acid; chenodeoxycholic acid; ursodeoxycholic acid	Taurine	(*102, 105*)
PROCYONIDAE			
Raccoon, *Procyon lotor*	Cholic acid; deoxycholic acid	Glycine; taurine	(*106*)
Ringtailed coati, *Nasau nasau*	Cholic acid	—	(*30*)
MUSTELIDAE			
Weasel, *Mustela itati*	Cholic acid	—	(*99*)

TABLE I (*Continued*)

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
Marten, *Martes melampus melampus*	Cholic acid; deoxycholic acid	Taurine	(*100*)
Fish otter, *Lutra*	Cholic acid; deoxycholic acid	Taurine	(*99*)
VIVERRIDAE			
Kusimanse, *Crossarchus obscurus*	Cholic acid	—	(*30*)
FELIDAE			
Felis leo	Cholic acid	Taurine	(*107*)
Leopard, *Felis pardus*	Cholic acid	Taurine	(*87*)
Domestic cat	Cholic acid	Taurine	(*108*)
Cheetah, *Acinonyx jubatus*	Cholic acid	—	(*30*)
OTARIIDAE[e]			
"*Otaria ursina*"	Cholic acid; phocaecholic acid	Taurine	(*108*)
Steller sea lion, *Eumetopias jubata*[f]	Cholic acid; acid (?) $C_{27}H_{46}O_6$	—	(*109*)
Californian sea lion, *Zalophus californianus*	Phocaecholic acid	—	(*33*)
ODOBENIDAE[e]			
Walrus, *Odobenus rosmarus*	Cholic acid; phocaecholic acid[g]	Taurine	(*110*)
PHOCIDAE (seals)[e]			
Phoca barbata *Phoca groenlandica* *Phoca foetida* *Cystophora cristata*	Cholic acid; phocaecholic acid[g]	Glycine; taurine	(*110*)
Phoca hispida	Phocaecholic acid	—	(*59*)
Gray seal of the Baltic Sea	Cholic acid; phocaecholic acid; chenodeoxycholic acid; 3α,7α,12α,23-tetrahydroxycholanic acid	—	(*32*)
Leopard seal, *Hydrurga leptonyx*	Cholic acid; phocaecholic acid; Ohta's acid[h]; 3α,7α,12α,23-tetrahydroxycholanic acid	—	(*33*)
ELEPHANTIDAE			
Elephant	Cholic acid; deoxycholic acid	—	(*111*)
EQUIDAE			
Domestic horse	Cholic acid	—	(*30*)

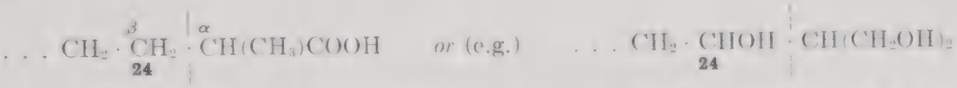
TABLE I (*Continued*)

Animal (Class or Sub-class, Order, Family, Species)	Compounds isolated from the bile	Nature of conjugation	References
SUIDAE			
Domestic pig	(?) Cholic acid; hyocholic acid; chenodeoxycholic acid; hyodeoxycholic acid; 3α,6β-dihydroxycholanic acid; 3β,6α-dihydroxycholanic acid; 3α-hydroxy-6-oxocholanic (or allocholanic) acid; lithocholic acid	Glycine; taurine	(*30*, *37a*, *38*) (*39*, *40*, *42*, *43*) (*44*, *45*, *48*) (*54*)
Wild pig, *Sus leucomystax*	Hyodeoxycholic acid	Glycine	(*112*)
Abyssinian wild pig	Hyocholic acid; chenodeoxycholic acid; hyodeoxycholic acid	Glycine; taurine	(*113*)
Wart hog, *Phacochoerus aethiopicus*	Cholic acid; (probably) hyodeoxycholic acid	Glycine; taurine	(*11*, *30*, *113*)
HIPPOPOTAMIDAE			
Hippopotamus amphibius	Cholic acid; chenodeoxycholic acid	—	(*114*, *115*)
BOVIDAE			
Eland, *Taurotragus oryx*	Cholic acid	—	(*30*)
Water buffalo, *Bubalus bubalus*	Cholic acid; deoxycholic acid; chenodeoxycholic acid	—	(*116*)
Antelope	Cholic acid; deoxycholic acid	—	(*77*)
Domestic ox	Cholic acid; deoxycholic acid; chenodeoxycholic acid; lithocholic acid; 3α-hydroxy-12-oxo-, 3α,12α-dihydroxy-7-oxo; 7α,12α-dihydroxy-3-oxo-, and 3α-hydroxy-7,12-dioxocholanic acids; sterocholic acid[h]; sapocholic acid[h]	Taurine; glycine	(*4*, *77*)
Musk ox, *Ovibos moschatus*	Cholic acid; deoxycholic acid	Taurine; glycine	(*117*)
Domestic goat	Cholic acid; deoxycholic acid; chenodeoxycholic acid	Taurine; glycine	(*77*, *118*)
Domestic sheep	Cholic acid; deoxycholic acid; chenodeoxycholic acid	Taurine; glycine	(*50*, *77*)

information about microbial artifacts is now available, and this has done much to clarify the position of some biliary constituents. It is considered below.

B. Evolution of Bile Salts

It seems abundantly clear that evolution of bile salts has taken place. The subject has been briefly reviewed (*11*), and it will suffice here to point out that the stages seem to be similar to some of those occurring in a modern vertebrate in the route cholesterol → bile salts (*48, 48a*). Cholesterol is probably converted to (A/B *cis*) primary alcohols with molecules containing additional α-orientated hydroxyl groups in the "bile salt" positions; such alcohols are found, conjugated with sulfate and functioning as bile salts, in Selachii, Teleostei, and Amphibia. Next, the C-27 terminal primary alcoholic group (e.g., structure II) is oxidized to produce hydroxylated coprostanic acids (e.g., III) and such are found (conjugated with taurine) in Amphibia and more "primitive" reptiles. Side-chain β-oxidation



$$\ldots\ \mathrm{CH_2}\cdot\underset{\mathbf{24}}{\overset{\beta}{\mathrm{CH_2}}}\ \Big|\cdot\overset{\alpha}{\mathrm{CH(CH_3)COOH}}\qquad \textit{or}\ \text{(e.g.)}\qquad \ldots\ \mathrm{CH_2}\cdot\underset{\mathbf{24}}{\mathrm{CHOH}}\ \Big|\cdot\mathrm{CH(CH_2OH)_2}$$

gives the C_{24} bile acids commonly found in all "modern" vertebrates. Glycine conjugates of the C_{24} acids are perhaps still more modern, being found only in mammals and possibly only in the Eutheria.

No information, for example about the relative physiological efficiency of the different types of bile salts, is available at present to provide material for speculation as to the selection pressures or other forces which may have caused this type of evolution. It appears to have gone *pari passu* with "modernization" in most orders, and might be expected to lead to the presence in all surviving vertebrates of the C_{24} acids or their evolutionary successors.

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[a] Names of Japanese fishes will be found in catalogues from Tokyo (*119*).

[b] Ohta's acid (allo(5α)cholic acid); see text (Section I,B,3).

[c] May be an artifact, derived from Δ^4-7α-hydroxy-3-oxocholenic acid.

[d] Not confirmed, as an individual substance, by later work.

[e] The seals, sea lions, and walruses are grouped in the suborder Pinnipedia.

[f] In a review (*1*) this animal, described originally (*109*) as *Eumetopia tubata* was incorrectly listed as a fish.

[g] The bile was reported to yield also "α-phocaecholic acid" (what is here called "phocaecholic acid" being designated "β-phocaecholic acid"). Recent work on the "α" acid of Hammarsten (*110*) has shown that it contains 3α,7α,12α,23-tetrahydroxycholanic acid and also allocholic acid (*32, 33*).

[h] Probably not bile acids in the sense used here.

It is an interesting possibility that biochemical "recapitulation" may be taking place when bile salts are formed from cholesterol in modern animals.

C. Microbial Artifacts in the Bile

During the enterohepatic circulation, products formed by microbial action in the intestinal tract (including the cecum) are absorbed and excreted in the bile. Such products in the gall bladder will reflect imperfectly the actions of the liver enzymes which presumably make the bile salts from sterols. Their value as species characters is uncertain and must be assessed in every individual case. Much valuable work has been done, mainly by S. Bergström and his colleagues at Lund and Stockholm, on studies of the action of intestinal microorganisms during the enterohepatic circulation. A chief method of the Swedish workers has been to establish a fistula and then to compare fistula bile with that taken from the gall bladder. Then, C^{14}- or H^3-labeled steroids can be administered and the labeled products identified in the bile. Bergström *et al.* (*48*) have reviewed their work and that of others. Some of the main conclusions are as follows:

(*a*) Deoxycholic acid has been shown in all species studied (rat, rabbit, man, two species of Boidae) to be made from cholic acid by microbial action in the intestine. Man and the rabbit re-excrete deoxycholic acid, which is found in the gall bladder (in the rabbit in much the greatest concentration of the bile acids); the rat hydroxylates the deoxycholic acid to re-form cholic acid and the Boidae put in a 16α-hydroxyl group to give pythocholic acid. Thus, part of the cholic acid in rat bile is of "secondary" origin, like pythocholic acid. The specific character of the Boidae is the possession of liver enzymes which effect 16α-hydroxylation of deoxycholic acid, and would presumably not be manifest if this substance was not made by intestinal microorganisms. It does, however, look as if the rat and the Boidae have evolved mechanisms (7α- and 16α-hydroxylation, respectively) for converting deoxycholic acid to a trihydroxycholanic acid, and presumably such evolution has taken place as a result of selection pressure. Man and the rabbit have no such rehydroxylation mechanisms. It would be interesting to know if other snakes, having cholic and not pythocholic acid, can carry out, like the rat, 7α-hydroxylation of deoxycholic acid.

(*b*) The characteristic bile acids of the pig are derived from hyocholic acid. Hyodeoxycholic acid is formed from this in the intestine, as are 3α-hydroxy-6-oxocholanic acid and probably 3α,6β- and 3β,6α-dihydroxycholanic acids.

(*c*) All the keto acids investigated, as well as ursodeoxycholic acid, have been shown to be probably microbial artifacts. This disposes of the view, at one time held, that the keto acids are intermediates in bile salt formation and possibly of reasons for thinking that 3α-hydroxy-7-oxocholanic acid (formerly called nutriacholic acid) is a character specific to the coypu, or that ursodeoxycholic acid is characteristic of bear's bile. It has, however, been suggested (*92a*) that 3α-hydroxy-7-oxocholanic acid in guinea pigs is formed "without action of enteric microorganisms."

The status of 3α,7β,12α-trihydroxycholanic acid is uncertain, as is that of allocholic (Ohta's) acid. The distribution of 23-hydroxylated C_{24} acids (phocaecholic, etc.) suggests that 23-hydroxylation is a true species character.

No work has been reported on the possible effect of intestinal microorganisms on any bile salts other than those containing C_{24} acids.

D. USE OF THE CHEMICAL NATURE OF BILE SALTS AS A SPECIES CHARACTER

If the qualifications mentioned above are borne in mind, it seems possible that consideration of the chemical nature of bile salts may prove useful in biological studies. In cases in which morphology is an uncertain guide the chemical evidence may be found valuable. It has been cited recently (*94*) in a comparison between the West African cutting-grass (cane rat) *Thryonomys swinderianus* and the South American coypu *Myocastor coypus*. These rodents are so closely similar that at one time Simpson (*120*) placed them both in the superfamily Octodontoidea. However, Wood (*121*) and others disputed this, on paleogeographical grounds. An examination of the bile salts showed that *Thryonomys* conjugated only with taurine, whereas *Myocastor*, like the guinea pig, had mainly glycine conjugates. It was felt that this evidence supported Wood's rather than Simpson's (1945) views. Similar comparisons might well be made in other instances in which systematists disagree about morphological evidence.

Light may possibly be thrown on evolutionary history by bile salt examinations. For example, if a lizard could be found which hydroxylated deoxycholic acid as do the Boidae (see above), there might be an *a priori* case for considering it as a limbed ancestor to these snakes. Again, a study of the occurrence of cholic acid and of 6α-hydroxylated bile acids among the Suidae might help in unraveling the history and relationships of this family. Many similar examples will occur to biologists, and help might reasonably be expected in those cases where the bile salts show unusual or rare features. It seems obvious, however, that in many, perhaps most, "modern" species the bile salts are generalized and do not show qualitative chemical features which appear helpful. In some such cases, quantitative studies may be of value, and some preliminary work on these lines has been done among the Primates (*82*).

It is also clear that bile salts are not always what they seem, and closer study may reveal, as in the rat, liver enzymes which constitute hitherto unsuspected species characters. In any case, enzymatic studies are likely eventually to be more valuable then chemical investigations on gall bladder (or even fistula) bile, and, for this reason, discovery of a liver preparation that will make bile salts *in vitro* would do much to increase understanding of the subject.

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CHAPTER 5

Distribution of Phospholipids

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I. Introduction

The phospholipids form a rather heterogeneous group of compounds which have been classed together principally on the basis of solubility. Except for this similarity in solubility and the fact that many of them contain structural units besides phosphorus which are the same or similar, there is little reason why they should be considered collectively. Very little is known of their metabolic functions, and nothing of what is known suggests that the individual phospholipids bear any relationship to one another in this respect. It does seem significant, however, that as a class of compounds, the phospholipids have been found in every cellular organism which has been examined for them and are probably a constituent of every living cell. Yet, the distribution of any given type of phospholipid varies not only from species to species, but also from one tissue to another in the same organism. What significance can be placed on this disparity of distribution is not clear, but it does suggest that perhaps the field of comparative biochemistry of phospholipids may provide important information concerning the possible function of the phospholipids.

It is with this in mind that certain privileges have been exercised in the selection of material for inclusion here. There is an abundance of literature relating to the occurrence of phospholipids in various animals, plants, and microorganisms. Often nothing more is reported than the per cent weight or molar concentration of phospholipids or lipid phosphorus. Because of the importance of stressing the individuality of the different phospholipids as metabolic entities, in general, data of this type have not been included. Emphasis has been laid as far as possible on studies which distinguish to some degree the various types of phospholipids.

The fractionation of complex mixtures of phospholipids is difficult, and it has been only in comparatively recent times that even a start has been made toward the characterization of all the phospholipids of a given tissue. It is not surprising then that some methods of analysis in the past have been based on erroneous concepts and simplifications. Older data presented here have been interpreted in the light of present knowledge, and limitations have been pointed out when thought necessary. No attempt to give a historical presentation has been made, and emphasis has been placed on the most recent data available. It is hoped that any ambiguity which might arise from the nomenclature used, past and present, has been eliminated by the definition of terms included below in the discussion of structure.

II. Structure of the Glycerophosphatides

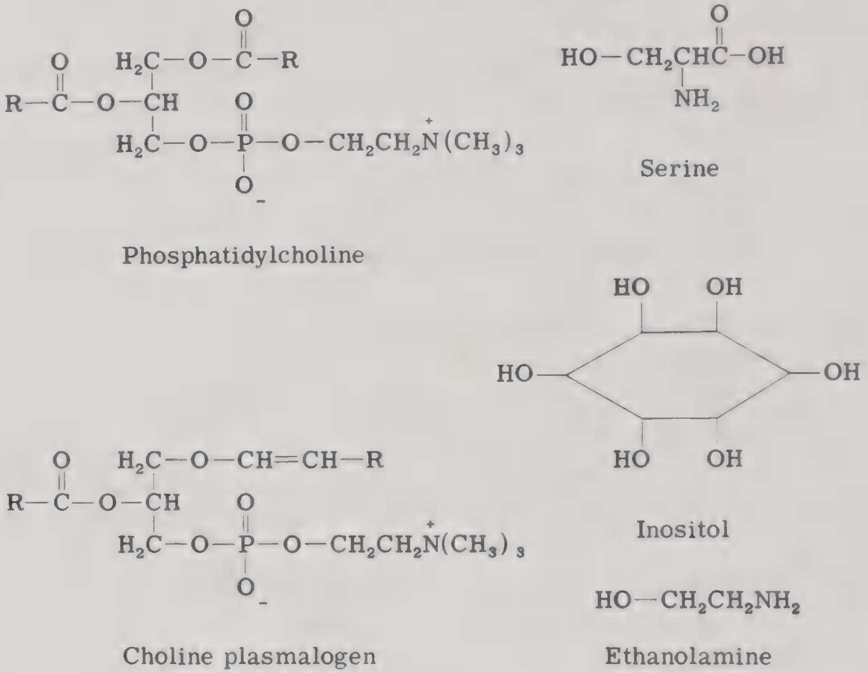
Regardless of source, those phospholipids which have been characterized are similar in structure and may be classified into two major groups, the glycerophosphatides and sphingophosphatides. The glycerophosphatides are the more common; and as their name implies, all contain glycerophosphoric acid as a basic unit of structure. Further, from the evidence available it is apparent that only L-α-glycerophosphoric acid is present. In other words the phosphate moiety is esterified to the primary hydroxyl group of glycerol; and as related to the corresponding 3-phosphoglyceraldehyde, the glycerophosphoric acid has the L-configuration. Reports in the older literature of β-phosphoglycerides and analyses given of mixtures of α- and β-glycerophosphatides are generally based on the presence of α- and β-glycerophosphoric acid in hydrolyzates of phospholipids. It is now known that β-glycerophosphoric acid is formed during hydrolysis by migration of the phosphate radical from the α-position. In no case has the natural occurrence of a β-phosphoglyceride in phospholipids been unequivocably demonstrated. The evidence for the L-α-configuration of the glycerophosphatides has been reviewed by Lovern (*1*) and by Baer *et al.* (*2*).

Two types of glycerophosphatides are widely distributed in nature. In the one, the glycerophosphoric acid is acylated with two long-chain fatty acids to give phosphatidic acid. The second type of glycerophosphatide, the plasmalogens, consist of an analogous series of compounds which have one long-chain alkyl group joined to the glycerol by a vinyl ether-type linkage and one acyl group. The relative position of the acyl and vinyl ether group is not completely resolved. This problem has been recently reviewed by Klenk and Debuch (*3*) and Dawson (*4*). In the past, phospholipids having a fatty aldehyde joined to glycerophosphoric acid by a hemiacetal- or acetal-type linkage have been described. Since these compounds were usually isolated after alkaline treatment of the phospholipids and the plasmalogen structure described above is known to give a hemiacetal or acetal on alkaline treatment, it is now generally considered that acetal phospholipids are artifacts. A possible exception to this is the reported isolation from a species of sea anemone of a choline plasmalogen which has no acyl group (*5*).

Although glycerophosphatides with only one fatty acid have been reported in preparations of phospholipids, e.g., by Marinetti *et al.* (*6*), Phillips (*7*), and Gjone and co-workers (*8*), they may result from enzymatic or chemical degradation of other phospholipids during isolation. Evidence for glycerophosphatides containing two fatty vinyl ether groups and one based on a fatty alkyl ether of glycerophosphoric acid has been presented by Marinetti *et al.* (*9*) and Carter and associates (*10*), respectively.

The final variation in structure is in a third component which is esterified to the glycerophosphoric acid to form a phosphate diester. The three nitrogenous constituents choline, ethanolamine, and serine are commonly found in the diacylglycerophosphatides whereas only the first two occur widely in the plasmalogens. Serine plasmalogen has been reported in brain (*11*). Inositol is also commonly found in the diacylglycerophosphatides but has been reported only twice as part of a plasmalogen (*12, 13*). Amino acids other than serine, peptides, and also glycerol have been reported as the additional constituent of glycerophosphatide by various investigators. The structure of these and other complex glycerophosphatides, including the polyglycerophosphatides, are discussed below in relation to their occurrence in specific tissues. The structure of the analogous choline diacylglycerophosphatide and plasmalogen and other substituents are shown in Fig. 1.

Since the simple diacylglycerophosphoric acid is called phosphatidic acid, the diesters are named with the prefix phosphatidyl-, for example, phosphatidylcholine. Many of these compounds have been isolated and characterized only quite recently, and their presence in fractions which were previously considered homogeneous has led to ambiguity in the nomenclature. In considering data from earlier studies, it is necessary to take these later findings into consideration. For all purposes here,


Phosphatidylcholine

Serine

Inositol

Choline plasmalogen

Ethanolamine

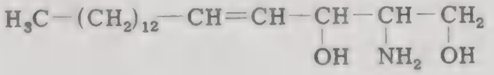
FIG. 1. Structures of representative examples of the two major types of glycerophosphatides shown as their choline analogs. Ethanolamine, serine, and inositol are all commonly found as substituents in place of choline. R in every case represents a long-chain alkyl group.

therefore, when a particular compound has been isolated or otherwise characterized, it will be called by its correct chemical name, e.g., phosphatidylcholine, etc. The term lecithin is used here to designate a lipid fraction that includes all the choline-containing phospholipids including the plasmalogens but excluding the choline phosphosphingosides. The term cephalin was originally applied to what is now known as phosphatidylethanolamine, and is still sometimes used in this context. Since in early work the cephalin fraction was actually a complex mixture of all the phosphoglycerides except lecithin, it is used here to designate all the nitrogenous glycerophosphatides except those containing choline and also includes the phosphoinositides. The prefix lyso- in every case refers to the analogous compound or fraction less one molecule of fatty acid.

One additional comment on the structure of the glycerophosphatides is of special importance to some of the comparative aspects. Any one of the types of phospholipids described above may produce on hydrolysis a fairly wide variety of fatty acids or, in the case of the plasmalogens, fatty acids and fatty aldehydes. There is evidence which suggests that there may be some limitation on the type of fatty acids found in given positions of the molecule (*14, 15*); yet, even taking this into consideration, a large number of different individual phospholipids exists for each of the various types described. A few individual phosphatidylcholines of known fatty acid content have been isolated. In other cases the fatty acids or fatty aldehyde of a given type of phospholipid have been analyzed and although the data only give a suggestion of the possible variety of individual phospholipids that may be present, they have in general been included. For the rest, it should be remembered that although phospholipids of different species may appear to be the same, because of their fatty acids they may in fact be completely different.

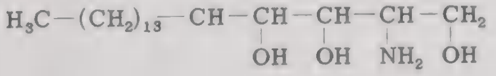
III. Structure of the Sphingophosphatides

The structures of the sphingophosphatides are based on the phosphate ester of the long-chain base sphingosine, D-*erythro*-2-amino-1,3-dihydroxy-4-*trans*-octadecene, or compounds of very similar structure. The work leading to the establishment of the structure of sphingosine has been reviewed by Carter *et al.* (*16*), and its structure and the structure of phytosphingosine (*17, 18*) are shown in Fig. 2. Besides these two, a dihydrosphingosine (*19*), the saturated analog of sphingosine, and a 20-carbon analog of phytosphingosine have been identified and characterized (*20*).



$$H_3C-(CH_2)_{12}-CH=CH-\underset{OH}{\underset{|}{CH}}-\underset{NH_2}{\underset{|}{CH}}-\underset{OH}{\underset{|}{CH_2}}$$

Sphingosine



$$H_3C-(CH_2)_{13}-CH-\underset{OH}{\underset{|}{CH}}-\underset{OH}{\underset{|}{CH}}-\underset{NH_2}{\underset{|}{CH}}-\underset{OH}{\underset{|}{CH_2}}$$

Phytosphingosine

FIG. 2. Long-chain fatty bases sphingosine and phytosphingosine, which are found in the phosphosphingosides of animals and plants, respectively.

The only fully characterized sphingophosphatide from animals is sphingomyelin. The elucidation of its structure, as shown in Fig. 3, has been actively pursued for several years and a good review of this work is given by Lovern (*1*). Although dihydrosphingosine has been shown to be a constituent of animal phospholipids (*19, 21*), direct evidence that it occurs as the analog of sphingomyelin, dihydrosphingomyelin, is still lacking. Evidence that a compound analogous to sphingomyelin but with the choline substituted by ethanolamine has been obtained (*22, 23*).

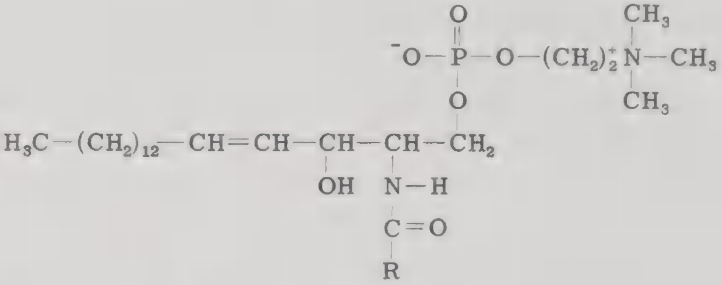


FIG. 3. Sphingomyelin, the most widely distributed sphingophosphatide in animal tissues.

The long-chain fatty bases phytosphingosine and C_{20}-phytosphingosine, as their names imply, are found in plants. Carter *et al.* (*24*) have recently almost completely resolved the structure of a phosphophytosphingoside found in soybeans and maize (Fig. 4), but except for this, little is known about the structure of the plant sphingophosphatides.

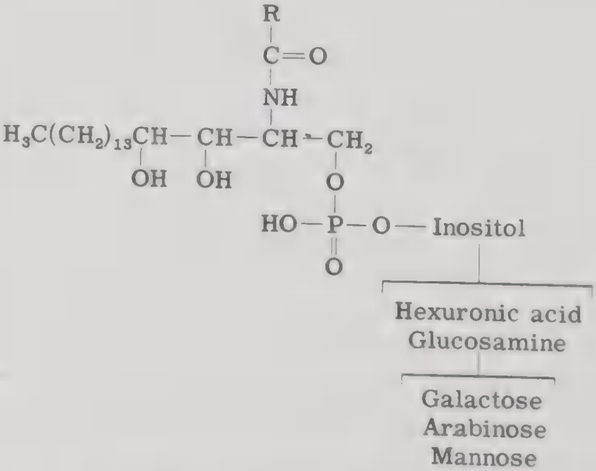


FIG. 4. The partially resolved structure of a phytosphingophosphatide found in soybeans and maize.

Analogs of sphingomyelin have not been isolated and probably do not occur.

In many cases it is difficult to evaluate the quantitative data given for sphingomyelin. Analyses have been carried out by a variety of methods, but almost all of these are subject to errors, especially when applied to tissues in which the phospholipids have not been fully characterized. The analyses included here should be accepted with the reservation that no obvious reason for discounting them is presently known. Further, although the variety of fatty acids found in sphingomyelins is more limited than that found in the glycerophosphatides, there is still a wide range of sphingomyelins, only a few of which have been identified.

IV. The Distribution of Phospholipids in Animal Tissues

A. Brain and Other Nervous Tissues

Brain, of all tissues which have been studied, has the greatest phospholipid content. From 25 to 30% of the dry weight of brain consists of phospholipids as compared to half this proportion in liver. Other parts of the nervous system, such as spinal cord or peripheral nerve, usually have as high concentrations of phospholipid. The diversity of phospholipids in brain is also greater than in any other animal tissue. Sphingomyelin (*18, 22, 25, 26*), phosphatidylcholine (*27*), phosphatidylethanolamine (*28*), and phosphatidylserine (*28, 29*) have all been isolated and characterized. Of the plasmalogens, that containing ethanolamine predominates (*30, 31*), but both a serine plasmalogen (*11*) and, probably, an inositol plasmalogen are present (*12*). Phosphatidylinositol (*32, 33*) is also present, and another phosphoinositide, the diphosphoinositide isolated by Folch (*34, 35*), is probably unique to brain. This latter compound contains inositol, glycerol, fatty acid, and phosphorus in a ratio of 1 : 1 : 1 : 2 and yields inositol diphosphate on acid hydrolysis. Most of these lipids have been isolated from or demonstrated in mammalian brains, and whether the complete range is found in all species is not certain. No simple method for estimating all the individual phospholipids of the brain is available.

A number of studies of the comparative aspects of phospholipids in brain and other nervous tissue have been carried out (*22, 36–38*). In general, only total phospholipid, lecithin, cephalin, and sphingomyelin have been determined. Data for a wide range of vertebrates have been summarized in Table I. A much more limited selection of data for vertebrate spinal cord and for invertebrate nervous tissue is given in Tables II and III, respectively. It should be understood that the method

TABLE I

DISTRIBUTION OF VERTEBRATE BRAIN PHOSPHOLIPIDS

Species		Total phospholipid (% dry wt.)	Cephalin (% dry wt.)	Lecithin (% dry wt.)	Sphingomyelin (% dry wt.)	Reference
Systematic name	Common name					
Class Mammalia						
Homo sapiens	Man[a]	31.0	20.2	5.0	5.9	(*39*)
Homo sapiens	Man[b]	35.1	9.3	7.3	7.8	(*22*)
Homo sapiens	Man[c]	26.5	14.1	5.7	6.7	(*22*)
Bos sp.	Ox[d]	23.0	15.9	3.5	3.3	(*40*)
Bos sp.	Ox[e]	19.4	11.0	5.6	2.9	(*40*)
Bos sp.	Ox[a]	26.3	14.3	7.0	4.9	(*39*)
Bos sp.	Ox[b]	20.6	11.1	5.6	3.9	(*22*)
Bos sp.	Ox[c]	27.4	15.4	4.9	7.1	(*22*)
Canis familiaris	Dog[a]	20.0	12.0	4.0	4.0	(*41*)
Felis domestica	Cat[a]	22.5	12.4	6.0	4.3	(*36*)
Lepus cuniculus	Rabbit[a]	26.0	14.6	7.3	4.5	(*36*)
Lepus sp.	Rabbit[b]	19.9	9.8	7.0	3.1	(*22*)
Lepus sp.	Rabbit[d]	23.3	17.2	3.2	3.0	(*40*)
Lepus sp.	Rabbit[e]	19.7	12.3	2.9	4.5	(*40*)
Cavis porcellus	Guinea pig[a]	23.0	13.2	6.6	4.4	(*36*)
Class Aves						
Gallus gallus	Hen[d]	18.6	12.2	3.3	3.1	(*40*)
Gallus gallus	Hen[e]	19.2	13.9	3.1	2.1	(*40*)
Larus argentatus	Herring gull[a]	20.3	12.0	6.8	2.3	(*36*)
Columba livia	Pigeon[a]	20.6	11.3	6.1	3.3	(*36*)
Class Reptilia						
Clemmys guttata	Spotted turtle[a]	23.8	13.4	7.9	2.7	(*36*)
—	Snake[d]	13.6	8.3	4.2	1.3	(*40*)

Class Amphibia						
Rana pipiens	Leopard frog[a]	18.9	7.6	8.2	2.0	(*36*)
Rana sp.	Frog[d]	14.9	7.9	5.0	1.6	(*40*)
Class Actinopterygii						
Amia calva	Bowfin[a]	9.4	5.5	3.5	0.5	(*36*)
Brevoortia tyrannus	Menhaden[a]	29.4	13.8	10.9	2.9	(*36*)
Perca flavescens	Yellow perch[a]	23.5	8.4	8.4	5.2	(*36*)
—	Mackerel[d]	22.2	10.1	7.3	4.4	(*40*)
Class Elasmobranchii						
Squalus acanthias	Dogfish[a]	20.5	10.8	6.5	3.5	(*36*)
Caracharias littoralis	Sand shark[a]	19.6	14.8	6.9	3.4	(*36*)
Raia erinacea	Skate[a]	22.8	11.2	6.5	5.6	(*36*)
Dasyatis sp.	Sting ray[a]	16.0	8.3	5.5	2.0	(*36*)

[a] Whole brain. [b] Brain cortex. [c] White matter. [d] Cerebrum. [e] Cerebellum.

TABLE II

DISTRIBUTION OF VERTEBRATE SPINAL CORD PHOSPHOLIPIDS

Species		Total phospholipid (% dry wt.)	Cephalin (% dry wt.)	Lecithin (% dry wt.)	Sphingomyelin (% dry wt.)	Reference
Systematic name	Common name					
Class Mammalia						
Felis domestica	Cat	28.8	14.9	4.7	9.6	(*37*)
Lepus cuniculus	Rabbit	32.9	16.7	5.6	11.5	(*37*)
Lepus cuniculus	Rabbit	26.3	16.5	3.1	10.9	(*40*)
Cavia porcellus	Guinea pig	26.5	13.9	5.8	7.1	(*40*)
Rattus sp.	Rat	26.0	13.0	6.9	3.1	(*41*)
Bos sp.	Ox	24.5	11.4	7.8	5.3	(*40*)
Class Aves						
Larus argentatus	Herring gull	22.1	12.6	5.8	3.7	(*37*)
Columba livia	Pigeon	18.6	8.6	3.8	6.1	(*37*)
Gallus gallus	Hen	23.5	15.4	3.8	4.6	(*40*)
Class Reptilia						
Clemmys guttata	Spotted turtle	27.7	12.9	7.4	7.4	(*37*)
Class Amphibia						
Rana pipiens	Leopard frog	20.9	7.7	8.7	5.1	(*37*)
Class Actinopterygii						
Amia calva	Bowfin	25.4	13.3	8.2	3.9	(*37*)
Brevoortia tyrannus	Menhaden	39.3	15.0	13.8	9.3	(*37*)
Perca flavescens	Yellow Perch	34.2	8.3	12.6	13.7	(*37*)
Class Elasmobranchii						
Squalus acanthias	Dogfish	25.6	12.1	7.4	6.1	(*37*)
Caracharias littoralis	Sand shark	30.3	15.5	9.6	7.3	(*37*)
Raia erinacea	Skate	31.0	12.8	9.6	8.6	(*37*)
Dasyatis sp.	Sting ray	30.2	15.0	8.5	6.5	(*37*)

TABLE III

The Distribution of Phospholipids in Some Invertebrate Nerve Tissue[a]

Species		Tissue	Total phospholipid	Cephalin	Lecithin	Sphingomyelin	Reference
Systematic name	Common name						
Loligo pealli	Atlantic squid	Pallial nerve	0.98	0.28	0.42	0.28	(*42*)
		Stellar nerve (whole)	0.33	← 0.06 →		0.07	(*42*)
		Stellar nerve axoplasm	0.18	← 0.15 →		0.03	(*42*)
		Central ganglia	3.21	1.64	1.17	0.40	(*42*)
Limulus sp.	King crab	Circumesophageal ring	1.36	0.22	0.61	0.51	(*38*)
		Ventral nerve cord	0.99	0.18	0.42	0.45	(*38*)
Libinia sp.	Spider crab	Ventral nerve cord	0.75	0.15	0.34	0.26	(*38*)
Apis mellifera	Honeybee	Central nerve system	17.0	12.0	2.0	1.5	(*43*)

[a] All values given as percentage of wet weight except those for *Apis mellifera* which are given as percentage dry weight.

of analysis used does not make any allowance for the occurrence of phospholipids different from the classic types and that the cephalin fraction is a mixture of probably no less than six different kinds of phospholipids. Important differences between species may be concealed within the data available.

As can be seen, there is usually little variation in the distribution of lecithin and cephalin in the brain of species in the same class. A striking exception is the over-all low level of phospholipid in the brain of the bowfin. No such generalization can be made about sphingomyelin. Although, with the exception of the fishes studied, the level of sphingomyelin does not vary greatly within each class, the concentrations in the various classes show a definite trend. The level in the brain of mammals is significantly higher than that found in most of the fishes and in the limited number of amphibians, reptiles, and birds studied. In agreement with this, Bieth and Mandel (*44*) found that the sphingomyelin concentration in the brain of one species each of fish (carp), tortoise, duck, fowl and of guinea pig, rat, cat, dog, and man increased in the order given. A similar but not as striking pattern is observed in the distribution of the phospholipids of spinal cord (Table II). It is interesting to note that the proportion of sphingomyelin in the phospholipids of the nervous tissue of invertebrates (Table III) is even lower than the proportion found in the submammalian vertebrates. In the honeybee, for which the data are directly comparable to the data in Table I, the level of sphingomyelin is also lower.

Before arriving at any final conclusions, one further aspect of the problem must be considered. At an early age the brain and other nervous tissues undergo myelinization. During this period the concentration of the phospholipids increases two- to threefold on the basis of wet weight. Of particular interest, however, is the much more rapid increase of sphingomyelin, a seven- to tenfold increase (*41*, *45*). Patterson *et al.* (*43*) noted that the phospholipid composition of the central nervous system of the bee was very similar to that of vertebrates before myelination, and McColl and Rossiter (*38*) have discussed the phospholipid concentration of invertebrate nervous tissue relative to the absence or presence of a myelin sheath. Certain differences between the sphingomyelin concentration of brain cortex and the more heavily myelinated white matter of brain of infants and adults have also been pointed out (*22*, *45*). It appears very likely that variations in phospholipid content of the brain of different species may be directly related to the relative amounts of certain cellular structures.

Although no comparative data are available, the fatty acid and fatty aldehyde composition of several brain phospholipid fractions have

TABLE IV

FATTY ACID COMPOSITION OF SOME BEEF BRAIN PHOSPHOLIPID FRACTIONS

Fraction	Fatty acid (% weight of total fatty acid)										Reference
	Saturated					Unsaturated					
	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	
Lecithin	Trace	29.0	9.0	1.2	—	1.2	48.0	7.3	4.3	—	*(27)*
Ethanolamine cephalin	Trace	9.7	9.5	1.5	0.9	2.3	34.7	15.1	24.4	1.7	*(11)*
Serine cephalin	—	3.2	32.7	—	—	—	51.6	7.1	5.4	—	*(11)*

been determined. The only fully characterized individual phospholipid is dipalmitoylglycerophosphorylcholine, which was isolated by Thannhauser *et al.* (*25, 46*). The fatty acids of the sphingomyelin of brain have been reported by Rennkamp (*26*) as 46% stearic, 34% nervonic plus lignoceric, 6% behenic, 2% each of palmitic and arachidic, and 10% of an unidentified hexacosenoic acid. Folch (*29*) reported that the brain phosphatidylserine contains equal quantities of stearic and oleic acids. Klenk and Böhm (*11*) have found a somewhat more complex fatty acid composition for serine- and ethanolamine-rich fractions of brain. Their data, with the data of Klenk and associates (*27*) for the lecithin fraction of brain, are given in Table IV. The fatty aldehydes from the whole phospholipid fraction of brain have been reported by Thannhauser *et al.* (*47*) to be entirely saturated, with palmitaldehyde predominating. In contrast to this, Klenk (*48*) and Leupold (*49*) report the presence of appreciable quantities of unsaturated C_{18} aldehyde.

B. Muscle

It has been shown that the phospholipid content is different in the three types of muscle, voluntary, cardiac, and involuntary or smooth. The work of Bloor (*50*) shows that while the total concentration of phospholipid in a given muscle of a wide variety of species varies, the relative amount of phospholipid in the three different types of muscle does not vary considerably. In general, cardiac muscle has a much higher phospholipid content than does skeletal or smooth muscle; and with the exception of some individual smooth muscles, skeletal muscle phospholipid concentration is higher than that of smooth muscle. A summary of data available is given in Table V.

Various phospholipids have been isolated from muscle and characterized. Almost all this work has been limited to mammalian heart muscle, notably beef heart, and the flesh of fish. Evidence for the presence of phosphatidylcholine and phosphatidylethanolamine in heart has been obtained by a number of workers, but a large part of the choline and ethanolamine occurs as plasmalogen (*51–55*). There is some evidence for phosphatidylserine (*54, 56*), but it has never been isolated on its own. Phosphatidylinositol has been isolated and well characterized (*54, 57*). Another well-characterized phospholipid of heart muscle is the polyglycerolphosphatide cardiolipin, which was first isolated by Pangborn (*58, 59*). Its structure, as shown in Fig. 5, is now well substantiated (*54, 60*).

The characterization of the phospholipids of the flesh of fish has been carried out on haddock by Olley and Lovern (*61*) and on cod by Garcia *et al.* (*62*). This work has been reviewed by Lovern (*63*). The presence of phosphatidylcholine and phosphatidylethanolamine is well established. Sphingomyelin, if present, occurs in trace amounts only. No evidence for phosphatidylserine was found in these two species, but both serine and threonine have been reported in the phospholipids of tunny muscle (*64*). Plasmalogens are present but apparently in much lower concentrations than in mammalian heart or skeletal muscle. Evidence for three different inositides has been obtained and also for two different polyglycerophosphatides. Apparently neither of these last two have the structure of beef heart cardiolipin, as fatty acid, glycerol, and phosphorus were found in a ratio of 4 : 2 : 1.

TABLE V

THE PHOSPHOLIPID CONTENT OF SKELETAL, CARDIAC, AND SMOOTH MUSCLE[a]

Species		Phospholipid (% of dry weight)		
Systematic name	Common name	Skeletal[b]	Cardiac	Smooth
Class Mammalia				
Homo sapiens	Man	—	7.0	3.5[c]
Canis familiaris	Dog	8.0	8.5	3.0[c]
Felis domestica	Cat	2.5	5.7	3.2[d]
Lepus sp.	Hare	1.7	9.1	4.4[c]
Sylvilagus floridanus	Wild rabbit	3.7	7.6	2.5[e]
Macropus rufus	Red kangaroo	2.4	6.7	—
Class Aves				
Gallus gallus	Hen	3.7	7.6	2.0[f]
Columbidae family	Pigeon	4.5	7.3	2.5[f]
Passer domestica	House sparrow	4.4	8.5	2.8[f]
Scops asio	Screech owl	3.6	6.2	3.5[f]
Class Amphibia				
Rana pipiens	Leopard frog	3.8	4.2	—
Class Reptilia				
Pseudemys scripta elegans	Scribe turtle	3.5	6.4	—
Alligator mississippiensis	Alligator	2.0	7.5	—

[a] Adapted from Bloor (*50*).
[b] Thigh.
[c] Uterus.
[d] Intestine.
[e] Stomach.
[f] Gizzard.

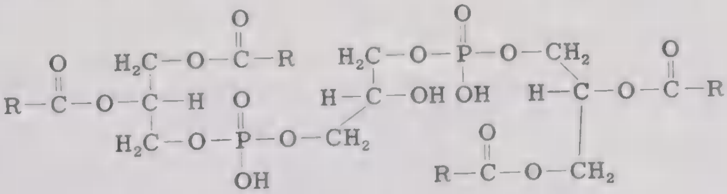


FIG. 5. The structure of beef heart cardiolipin.

Limited data are available on the fatty acid composition of muscle phospholipids. Gray and Macfarlane (*54*) report only minor amounts of saturated fatty acid in the cardiolipin of beef heart. Seventy per cent of the fatty acid was found to be linoleic acid. The remainder was made up of more or less equal amounts of palmitoleic, oleic, and linolenic. Pangborn reported that linoleic predominated in the cardiolipin she isolated. The remainder was made up of more or less equal amounts of palmitoleic, oleic, and linolenic. In agreement with the report of Faure and Morelec-Coulon (*65*), Gray and Macfarlane found that approximately 50% of the fatty acid of beef heart phosphatidylinositol was saturated. They found 46% stearic and 3% palmitic acid. Analysis of the unsaturated fatty acids showed approximately 42% oleic, 25% linoleic plus linolenic, 22% arachidonic, 9% C_{20} and C_{22} unidentified polyene acids, and 2% palmitoleic. Gray (*66*) has analyzed the fatty acids of phosphatidylcholine, phosphatidylethanolamine, and the choline and ethanolamine plasmalogens of beef heart. His data, summarized in Table VI, are in good accord with those reported by Klenk and Krickau (*67*) for phosphatidylcholine and choline plasmalogen from the same source. Gray (*66*) also reports that the aldehydes from beef heart choline plasmalogen are essentially all saturated, which is what Klenk and co-workers (*68*) have found for horse flesh and beef heart.

TABLE VI

FATTY ACID COMPOSITION OF BEEF HEART GLYCEROPHOSPHATIDES[a]

Fatty acid	Molar per cent of total fatty acid			
	Phosphatidylcholine	Phosphatidylethanolamine	Choline plasmalogen	Ethanolamine plasmalogen
Saturated	43.0	46.0	3.5	7.0
Unsaturated				
Oleic	31.4	17.7	38.6	17.3
Linoleic	18.3	7.3	37.6	31.6
Linolenic	4.2	0.6	7.7	10.2
Arachidonic	2.9	22.4	9.7	21.4
C_{20} Polyene	Trace	6.2	2.9	11.5
Total unsaturated	57.0	54.0	96.5	93.0

[a] From Gray (*66*).

C. BLOOD

The characterization of the individual phospholipids of blood has not been extensively pursued; however, it appears certain that at least in the plasma and erythrocytes the lipids are of the classic types. In the plasma, of all those species that have been studied, phosphatidylcholine predominates. Sphingomyelin is present generally at a concentration of 14–20% of the total phospholipid. In mammals, the cephalin concentration is characteristically low, usually less than 10% of the total phospholipid, and in some cases has been reported absent. Analyses of the phospholipids of chicken and turkey plasma consistently show a higher proportion of cephalin, 16–20%. The data for the distribution of cephalin, lecithin, and sphingomyelin for a limited range of species are given in Table VII. Obviously, more species of both mammals and birds must be studied to determine how significant the differences noted above may be. The extension of the study to fish, reptiles, and invertebrates should also be of particular interest.

TABLE VII

THE DISTRIBUTION OF PHOSPHOLIPIDS IN PLASMA AND SERUM

Species	Total phospholipid	Per cent of total phospholipid			Reference
		Cephalin	Lecithin	Sphingomyelin	
Class Mammalia					
Human plasma[a]	235	3	←——9.7——→		*(69)*
Human plasma[a]	153	28	55	12	*(70)*
Human plasma[b]	2.6	4.9	←——94.2——→		*(71)*
Human serum[b]	3.2	4.6	76.2[c]	19.0	*(7)*
Human serum[a]	140	7.9	52.8	39.3	*(72)*
Human serum[b]	2.7	5.5	78.9	14.9	*(73)*
Dog plasma[a]	255	5.0	←——95——→		*(69)*
Dog plasma[b]	3.8	6.3	←——92.2——→		*(71)*
Dog serum[a]	365	6.0	78.9	15.1	*(72)*
Beef serum[a]	79	3.2	68.2	28.9	*(72)*
Pig serum[a]	96	3.1	75.0	21.9	*(72)*
Class Aves					
Hen plasma[a]	162	21.3	64.9	13.8	*(74)*
Turkey serum[a]	241	20	←——78——→		*(72)*

[a] Total phospholipids in milligrams per 100 ml. and distribution of phospholipids in per cent of total phospholipids.

[b] Total phospholipid in millimoles per liter and distribution in molar per cent of total phospholipid.

[c] Phosphatidylcholine plus lysophosphatidylcholine.

The exact composition of the cephalin fraction is not known, but analyses of serine and ethanolamine by Artom (*70*) and Axelrod *et al.* (*71*) suggest that both phosphatidylethanolamine and phosphatidylserine are present. Direct evidence for the presence of phosphatidylinositol is also lacking; however, Taylor and McKibben (*75*) have reported the presence of inositol in the plasma phospholipids of dog, rabbit, and man. Both choline and ethanolamine plasmalogen have also been reported in plasma (*76*). No serine plasmalogen was detected. Dawson (*77*) has recently developed an analytical technique for the determination of all of the classic-type phospholipids in a tissue. This technique is based on the analytical determination of the deacylated compounds after separation by paper chromatography and accordingly provides good evidence for the occurrence in the tissue of the phospholipids concerned. Data obtained by this technique on the plasma of a number of mammals (Table VIII) are in general agreement with the data for mammals given in Table VII.

TABLE VIII
DISTRIBUTION OF PHOSPHOLIPIDS IN THE PLASMA OF VARIOUS SPECIES[a]

Fraction	Phospholipid phosphorus (% of total lipid phosphorus)					
	Human	Pig	Horse	Ox	Sheep	Goat
Phosphatidylcholine[b]	74.5	79.0	83.2	82.0	70.3	88.0
Phosphatidylethanolamine	1.8	2.2	0.5	—	2.4	0.5
Phosphatidylserine	—	—	—	—	—	—
Sphingomyelin	12.4	10.3	10.4	10.8	15.8	9.0
Choline plasmalogen	3.8	2.7	1.5	6.4	2.1	2.0
Ethanolamine plasmalogen	—	—	1.0	—	—	—
Serine plasmalogen	—	—	—	—	—	—
Phosphatidylinositol	1.4	2.2	—	2.4	—	1.7
Phosphatidic acid	—	—	—	—	0.9	0.4
Polyglycerophosphatide	—	0.5	—	0.7	1.6	—
Unidentified	1.3	0.6	0.7	2.0	0.6	0.8
Recovery (% of total lipid P analyzed)	95.2	97.5	97.3	104.3	93.7	102.4

[a] From Dawson and Lindsay (*78*).
[b] Includes lysophosphatidylcholine.

Except for plasmalogen, the same types of phospholipids that occur in plasma also apparently occur in erythrocytes. Not so large a variety of species have been examined; nevertheless, differences in distribution have been detected between the erythrocyte phospholipids of most ruminants and other mammals. Turner and co-workers (*79*) report that, while in the red cells of the dog, guinea pig, and dingo the ratio of lecithin to sphingomyelin is quite high, in goats, sheep, and oxen lecithin is absent or present only in traces. The ratios of lecithin to sphingomyelin in the red cells of man, cats, rabbits, dromedary, and guanaco were found to be intermediate between those of the other two groups. In confirmation of this, Hanahan *et al.* (*80*) have reported

TABLE IX

DISTRIBUTION OF PHOSPHOLIPIDS IN ERYTHROCYTES AND ERYTHOCYTE GHOSTS OF VARIOUS SPECIES[a]

Fraction	Human		Pig	Horse	Ox		Sheep	Goat
	Cell ghosts	Whole cells	Cell ghosts	Cell ghosts	Cell ghosts	Whole cells	Cell ghosts	Cell ghosts
Phosphatidylcholine	29.2	36.4	24.8	36.4	9.8	7.0	7.7	9.8
Phosphatidylethanolamine	14.4	17.6	26.4	6.8	9.0	6.2	5.4	6.8
Phosphatidylserine	11.7	5.1	16.6	8.8	14.0	15.4	12.6	14.2
Sphingomyelin	17.6	16.2	14.0	27.8	39.7	39.2	42.5	34.4
Choline plasmalogen	3.8	5.7	5.4	5.6	7.0	5.3	9.0	6.8
Ethanolamine plasmalogen	4.0	5.6	2.1	3.5	2.7	1.1	2.1	3.4
Serine plasmalogen	0.8	0.3	1.6	0.7	0.6	2.2	1.1	2.0
Phosphatidylinositol	1.6	—	3.2	3.6	3.5	1.8	3.6	5.8
Phosphatidic acid	1.4	1.2	0.8	0.5	0.6	0.3	0.4	0.2
Polyglycerophosphatide	—	—	—	0.6	0.5	0.3	0.4	1.2
Unidentified	3.2	2.6	1.0	2.2	2.6	1.1	2.6	2.2
	87.7	90.7	95.9	96.5	90.0	79.9	87.4	86.8

[a] Adapted from Dawson and Lindsay (*78*). Values given as percentage of total lipid phosphorus.

that in human and pig red cells the ratio of phosphatidylcholine to sphingomyelin is 1.5 : 1, but that no phosphatidylcholine can be detected in the red cells of beef. They have also demonstrated the presence of phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol in the red cells of all three of these species. No plasmalogen was detected. Dawson and Lindsay (*78*) have carried out analyses of the phospholipids of the erythrocyte ghosts of three ruminants and three nonruminants (Table IX). The different distribution of phospholipids in ruminant and nonruminant red cells is also found in the ghosts. This more detailed analysis also reveals that besides the significantly higher sphingomyelin and lower phosphatidylcholine content in ruminant erythrocytes, there is also a lower phosphatidylethanolamine content in the red cells of ruminants and of the horse. It is suggested that this may be characteristic of the herbivores. The data of Burt and Rossiter (*81*) for the erythrocytes of rabbits and of Formijne *et al.* (*82*) and Hack (*73*) for human red cells are also in line with the findings reported above.

Turner (*83*) has suggested that the failure of snake venom to cause lysis of red cells of ruminants is related to the absence of lecithin; however, there is still no satisfactory explanation of why the red cells of the camel should be resistant to lysis by snake venom yet contain lecithin.

D. LIVER

No comparative study of the distribution of liver phospholipids in different species has been made, and characterization of the individual phospholipids of liver is limited to a few mammals, in particular ox and rat (*84, 85*). For an organ with as diverse activities as the liver, the range of phospholipids is quite simple. A little over 50% of the phospholipid is phosphatidylcholine. Phosphatidylethanolamine (*84, 86*) and smaller amounts of phosphatidylserine and phosphatidylinositol (*85, 87, 88*) and sphingomyelin (*84*) are present. At least in rat and sheep liver, plasmalogens are present in only trace amounts (*77, 89*). Other phospholipids present in relatively small amounts are phosphatidic acid (*90*) and polyglycerophosphatides similar to cardiolipin of heart. This has been isolated from dog liver by McKibbin and Taylor (*91*), rat liver by Dawson (*87*), and beef liver by Faure *et al.* (*88*). The compounds from dog and rat liver have a glycerol : fatty acid : phosphorus ratio of 3 : 2 : 3, and that from beef has a ratio of 3 : 4 : 2. Whether this represents a true species difference has not been resolved. The distributions of these phospholipids in liver of rat and sheep are tabulated with other organs at the end of the section on animal phospholipids.

Among other phospholipids that have been reported in rat liver but are not yet well characterized is one containing glutamic acid (*92*) and two which have amino nitrogen : phosphorus : fatty acid ratios of 1 : 2 : 2 and 2 : 1 : 1, respectively (*93*). Bile, which is of course a secretion of the liver, contains only one phospholipid, namely, lecithin (*94*, *95*).

Hanahan (*14*) has reported that the fatty acids of the lysolecithins produced from the liver lecithins of beef, rabbit, rat, guinea pig, and dog by the action of lecithinase A of snake venom are all saturated. On the basis of the specificity of lecithinase A of snake venom for the α' fatty acid of lecithin (*96*), this was interpreted to indicate an asymmetric distribution of fatty acid in liver lecithin. A similar asymmetry has been reported by Borgström (*97*) and Johnston and Nakayama (*98*) for the lecithin of bile. Recently some doubt has been cast on the specificity of lecithinase A (*15*, *99*), and this will have to be resolved before the asymmetry of lecithin can be fully resolved. More recently, Dittmer and Hanahan (*100*) have determined the fatty acid compositions of the three major phospholipids of rat liver. The data, including a division of the fatty acids of the lecithin on the basis of the action of lecithinase A, are given in Table X. It is interesting to note that the fatty acids of the phosphatidylinositol of beef liver are reported by Faure *et al.* (*88*) to be a mixture of stearic acid and unsaturated fatty acids.

TABLE X

FATTY ACID COMPOSITION OF RAT LIVER PHOSPHOLIPIDS[a]

Phospholipid	Linoleic	Arachidonic	Docosahexaenoic	Palmitic	Oleic	Stearic
Phosphatidylethanolamine[b]	10.1	23.6	9.6	20.9	10.6	23.2
Phosphatidylcholine released by lecithinase A	32.8	41.0	10.6	3.7	9.3	2.4
Lysolecithin	←	4.2	→	44.8	9.6	41.2
Phosphatidylinositol	4.0	38.2	9.1	7.9	5.3	37.9

[a] From Dittmer and Hanahan (*100*). Data reported in molar per cent of total fatty acid.

[b] Contains approximately 3% phosphatidylserine.

E. EGGS

Of animal tissues and products not described above, only eggs have been thoroughly enough investigated to warrant detailed discussion of their phospholipids. Most of this work has been done on chicken eggs; however, a comparative study by Masuda and Hori (*101*) gives the lecithin concentration as weight per cent of total phospholipid of an interesting range of species as follows: hen 69%, duck 75%, quail 74%, peacock 82%, salmon 90%, prawn 90%, cod 62%, carp 88%, halibut 72%, herring 42%, shark 100%.

For chicken eggs, Rhodes and Lea (*102*) report on a molar basis 73% phosphatidylcholine, 5.8% lysophosphatidylcholine, 15% phosphatidylethanolamine, 2.1% lysophosphatidylethanolamine, 2.5% sphingomyelin, and 0.6% inositol phosphatide. Plasmalogen is present in trace amounts, 0.9%; and there is evidence that a phospholipid containing threonine may be present. Subsequent to this report, Carter *et al.* (*10*) reported the isolation of a new phospholipid from egg. It was found to be an ethanolamine phosphate diester of an alkyl glyceryl ether (Fig. 6).

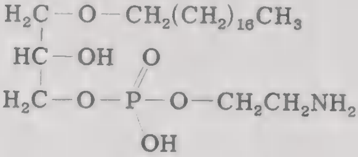


FIG. 6. *O*-Phosphoethanolamine derivative of batyl alcohol found in eggs.

Since a mild alkaline hydrolysis was used during isolation, it is possible that there is a fatty acid on the third hydroxyl group of the glycerol.

The fatty acid composition of egg phosphatidylcholine and phosphatidylethanolamine has been determined by Hawke (*103*). The fatty acid composition has been shown to vary with diet (*104*).

F. OTHER VERTEBRATE TISSUES

Of phospholipids not discussed above, the isolation of dipalmitoyllecithin from lung and spleen is of interest (*46*). The presence of phosphatidylcholine, -ethanolamine, and -serine in a wide range of tissues is suggested by the analyses of McKibbin (*105*), Artom (*70*), and Nojima and Utsugi (*106*) for choline, ethanolamine, and serine in the lipids from such organs as lung, spleen, kidney, testis, and intestine. Lipid inositol has been determined in pancreas and all the tissues listed above except spleen and testis (*75*).

Coincident to metabolic studies of phospholipids, evidence has been presented for the occurrence of phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, and phosphoinositide in mouse liver, kidney, brain, lung, heart, pancreas, and spleen (*107*), rat adrenal (*108*), and guinea pig adrenal medulla (*109*). Phosphatidic acid, phosphatidylcholine, -ethanolamine, and -serine have been identified in pigeon pancreas (*110*). Similarly, Freinkel has presented evidence for phosphatidic acid, phosphatidylcholine, -ethanolamine, -serine, and -inositol in thyroid (*111*, *112*).

TABLE XI
PHOSPHOLIPID COMPOSITION OF RAT TISSUE[a]

Fraction	Liver	Kidney	Spleen	Lung	Heart
Phosphatidylcholine[b]	58.1	33.7	44.4	34.3	37.0
Phosphatidylethanolamine[c]	20.4	17.6	10.9	9.1	22.7
Sphingomyelin	8.2	20.8	17.4	26.0	12.2
Phosphatidylserine	2.0	12.0	13.0	12.8	7.0
Phosphatidylinositol	1.9	2.7	2.4	2.6	2.3
Polyglycerophosphatide	3.4	5.4	4.3	5.3	10.2
Lysophosphatidylcholine	3.4	4.0	4.1	5.1	4.0
Undetermined	—	—	—	1.6	—

[a] From Marinetti *et al.* (*125*). Values given as percentage of total phospholipid phosphorus.

[b] Includes choline plasmalogen.

[c] Includes ethanolamine plasmalogen.

Plasmalogens have been demonstrated in adipose tissue of rats (*89, 113*) and fowl (*114*) and as important constituents of spermatozoa (*115, 116*). The analyses by a number of workers combine to show that, besides the organs mentioned previously, plasmalogens are probably present in kidney, adrenal, thyroid, lung, spleen, stomach, ovary, testis, uterus, and colon (*117–121*). Sphingomyelin analyses have been re-

TABLE XII
THE DISTRIBUTION OF PHOSPHOLIPIDS IN VARIOUS TISSUES OF SHEEP[a]

Fraction	Phospholipid phosphorus (% total lipid phosphorus)					
	Brain	Kidney	Liver	Skeletal muscle	Lung	Heart muscle
Total phospholipid (mg. P/gm. wet tissue)	2.20	1.22	1.94	0.55	1.02	0.91
Phosphatidylcholine	38.6	36.4	55.2	39.9	48.5	27.5
Phosphatidylethanolamine	17.5	20.0	26.5	14.7	14.0	19.1
Phosphatidylserine	16.0	8.2	4.4	3.8	9.9	3.5
Phosphatidylinositol	2.2	4.2	6.1	2.6	2.6	3.8
Polyglycerophosphatide	1.1	6.7	3.2	7.0	0.8	14.5
Phosphatidic acid	1.0	Trace	Trace	—	0.5	—
Choline plasmalogen	—	1.1	—	2.3	0.8	11.4
Ethanolamine plasmalogen	11.0	5.3	0.2	6.6	6.2	6.7
Serine plasmalogen	0.4	Trace	—	—	—	—
Sphingomyelin	9.4	13.2	4.2	6.4	12.4	6.6
Unidentified	5.6	3.0	0.5	3.4	1.9	7.9
Recovery (% of total lipid P analyzed)	100.8	98.1	100.3	86.7	97.6	101.0

[a] From Dawson (*77*).

ported for several organs of humans and rats (*39*, *122*). The percentages of phospholipid occurring as sphingomyelin in human and rat are, respectively, in lung 21.8 and 14, brain 18.3 and 5.0, liver 3.9 and 2.6, kidney 9.0 and 14.0, spleen 9.9 and 14.1, and heart 4.9 and 4.9. The fatty acids of the sphingomyelin of lung have been identified as principally palmitic and lignoceric and of spleen sphingomyelin as palmitic, stearic, and lignoceric (*123*, *124*).

Finally, in order to give some suggestion of the distribution of the phospholipids in mammalian tissues, the data from two recent analyses are included in Tables XI and XII.

G. Phospholipids of Invertebrates

Of invertebrate phospholipids not mentioned above, the analyses of the phospholipids of the sea anemone *Gyrostoma* sp. by Rajagopal and Schonie (*126*) may be noted. They report on the basis of total lipid 55.1% lecithin, 4.4% sphingomyelin, and 17.3% cephalin. In sharp contrast, Bergmann and Landowne (*5*) report sphingomyelin and lysoplasmalogen as the only significant phospholipids in the sea anemone *Anthopleura* sp. The same workers report the presence of plasmalogen in the sponge *Sphecia spongia vesparis*. Plasmalogens are also a constituent of the round worm, *Ascaris lumbricoides*, and of the pyloric cecum of the starfish (*127*). Analyses by Fairbairn (*128*) show that approximately 45% of the body wall phospholipids of the round worm is plasmalogen. Choline, ethanolamine, and traces of serine are reported; this suggests a similarity to the phospholipids of vertebrate muscle. Completely divergent from vertebrate phospholipids are those of the tapeworm *Taenia saginata*, which consist of lecithin and inositide only (*129*). No lipid ninhydrin-reactive material could be detected. The isolation of dipalmitoyl lecithin from the larval tapeworm *Cysticercus fasciolaris* has been reported (*130*).

Étienne and Kahane (*131*) have isolated phosphatidylcholine from the limpet *Patella vulgata*. These workers also identified betaine in the lipids of this animal, but it is not certain that it is an actual part of a phospholipid. Finally in regard to invertebrates, the phospholipids of the fruit fly *Drosophila melanogaster*, which appear to be otherwise of the classic types, contain a number of different amino acids (*132*).

V. The Distribution of Phospholipids in Plants

A. Seeds

The amounts of phospholipids reported in the seeds of a variety of plants by different workers vary considerably. Unquestionably, the amount, and quite possibly the composition, varies with the source, time of harvest, and other factors. Accordingly, the establishment of species differences presents a formidable problem. In general, however, those seeds that have a high oil content also have a high phospholipid concentration. Soybean, cottonseed, and peanuts all have from 2 to 3.5% phospholipids whereas the seeds of those plants that are generally not used as a commercial source of oil (excluding the legumes) have less than 1%. Wittcoff (*133*) gives further information on the phospholipid content of seeds.

There is no information on the distribution of all the different phospholipids of a given seed or indeed of the exact nature of all the seed phospholipids. Early workers differentiated the phospholipids only on the basis of cephalin and lecithin fractions. Choline and ethanolamine are widely distributed, and phosphatidylcholine and phosphatidylethanolamine have been isolated from soybean (*134, 135*). Although serine has been found in the phospholipids of peanuts (*136*), wheat and rye germs (*137*), and soybeans (*138*), phosphatidylserine has not been isolated. There is some evidence that serine may be present as part of a complex phosphoinositide (*136*). Phosphatidylinositol has been isolated from peas (*139*), soybeans (*30, 140*), and wheat germ (*141*). It is also apparent that a lysophosphatidylinositol is present in soybeans (*32*). The presence of more complex phosphoinositides in maize and soybeans is indicated by the work of Carter *et al.* (*10*) and in peanuts by Malkin and Poole (*142*). The structure of the compound isolated by Carter *et al.* has already been given in Fig. 4. This lipid probably accounts for the galactose, mannose, and arabinose reported in the inositides of corn and soybean by Scholfield *et al.* (*143*). The structure of the inositide isolated from peanuts by Malkin and Poole is not fully defined; however, it is probably a *N*-glycosyl derivative of the ethanolamine ester of phosphatidylinositol phosphate. The glycosyl unit contains L-arabinose and D-galactose in a molar ratio of 2:1. Phosphatidic acid, which has been reported to occur in wheat germ (*144*) and soybeans (*138*) may be produced by enzymatic hydrolysis during isolation.

The evidence of Lovern (*145*) for the occurrence of plasmalogen in seed phosphatides has been further substantiated by the reported identification of choline, ethanolamine, and inositol plasmalogens in peas (*13*).

B. VEGETATIVE TISSUES

The enzymatic degradation of plant phospholipids to phosphatidic acid during isolation has been established by Hanahan and Chaikoff (*146, 147*). Their data on phospholipids isolated from carrot roots and cabbage leaves with and without prior steaming to destroy enzymes, indicates that the naturally occurring lipids are probably of the classic types. Smith (*148*) showed that a similar production of phosphatidic acid occurs in rubber latex, and analysis of lipids isolated with boiling ethanol show that probably 79% of the natural phospholipid is lecithin. Subsequent work (*149*) showed that reducing sugar, inositol, and ethanolamine are also present in latex phospholipids.

Maruo and Benson (*150*) have identified α,α-diglycerol phosphate in mild alkaline hydrolyzates of the lipids of the alga *Scenedesmus* and have also now demonstrated by chromatographic techniques that it is a component of lipids from tobacco, sweet clover, and barley leaves (*151*). Their chromatographic demonstration of the presence of glycerylphosphoryl-choline, -ethanolamine, -serine, and -inositol in mild alkaline hydrolyzates of the phospholipids from these four sources suggests that the corresponding glycerophosphatides may be present. It should be noted that the composition of plant phospholipids they report have been based on the percentage of total radio activity from incorporated P^{32}. Since the specific activity of the phosphate in the different phospholipids was not shown to be the same, nor under the circumstances would they be expected to be the same, the data presented are only of qualitative value. A study by Kates (*152*) using techniques similar to those used by Benson and Maruo (*151*) suggests that the same range of phospholipids is also present in runner bean leaves. Finally, it has often been suggested that betaine can be substituted for choline in plant lipids. However, this has recently been investigated by Simenauer (*153*), who found no evidence for betaine in the phospholipids of the flower, root, and fruit of sugar beet *Beta vulgaris*, nor in the phospholipids of *Cicer arietinum*. Choline was identified in the phospholipids of all these sources.

C. Fungi

In early studies on the phospholipids of yeast, choline and ethanolamine were found in the phospholipids of *Saccharomyces cerevisiae* and *Torula utilis* in the molar ratios of 4:1 and 3:1, respectively (*154, 155*). In the latter, the presence of other phospholipids was suggested by the fact that 23% of the nitrogen could not be accounted for as either choline or ethanolamine (*155*). In contrast to this, the phospholipids of *Rhodotorula gracilis* have been reported to contain no ethanolamine, but serine was tentatively identified as a component. Both sphingosine and inositol were absent (*156*).

More recently, Hanahan and Jayko (*157*) reported the isolation of dipalmitoylglycerylphosphorylcholine from S. *cerevisiae*. Further work on the phospholipids of *S. cerevisiae* by Hanahan and co-workers (*84, 85*) indicate the presence of phosphatidylcholine, -ethanolamine, -serine, and -inositol. Small amounts of sphingomyelin and polyglycerophosphatide probably occur. There is also some evidence for lysophosphatidylethanolamine and lysophosphatidylserine, but this may have been formed during the isolation procedure.

The simplicity of the yeast phospholipid composition extends to the fatty acids, which are normal straight-chain acids. In both *S. cerevisiae* and *T. utilis*, saturated acids occur to the extent of 14–16% of the total (*158, 159*). The remainder of the fatty acids of *S. cerevisiae* are reported to be, primarily, oleic and palmitoleic (*158*) and in *T. utilis*, oleic and linoleic in a ratio of 3:1 (*159*).

In fungi other than yeast, choline and ethanolamine have been reported as constituents of the phospholipids of *Blastomyces dermatitidis* (*160*), *Aspergillus sydowi* (*161*), *Penicillium chrysogenum* (*162*), *Oïdium lactis* (*155*), and *Neurospora crassa* (*163*). A positive Molisch test indicates there may be carbohydrate in the phospholipids of the first of these and in *Monilia albicans* also (*164*). Of other constituents, serine and tyrosine have been reported in *P. chrysogenum* (*162*), and serine, 1-amino-2-methyl-2-propanol, and inositol in *N. crassa* (*163, 165*). In *A. sydowi* and *B. dermatitidis* (*161, 164*) the fatty acids have been reported to be of simple straight-chain types.

VI. The Phospholipids of Bacteria

The most striking aspect of the bacterial phospholipids is that one or more of the classic nitrogenous components is often missing. The principal exceptions are *Phytomonas tumefaciens*, in which the phosphospholipids are reported to yield equal amounts of choline and ethanolamine and a small amount of unidentified amino acid (*166*), and Bacillus "M" in which approximately 30% of the phospholipids are associated with choline, ethanolamine, and serine (*167*). Choline has been reported in the phospholipids of *Lactobacillus acidophilus;* however, it is a minor component and accounts for only a small part of the total nitrogen (*168*). No ethanolamine is found. Ethanolamine has been detected in hydrolyzates of phospholipids from *Escherichia coli* (*169, 170*), *Salmonella typhi* (*171*), *Mycobacterium marianum* (*172*), and *Vibrio cholerae* (*173*). Choline is absent from the lipids of all these organisms. Two other species of *Salmonella, S. ballerup* (*174*) and *S. paratyphi* (*175*), and two species of *Mycobacterium, M. phlei* (*176*) and *M. leprae* (*177*), and *Corynebacterium diphtheriae* (*178*) have all been reported to contain no lipid choline or ethanolamine.

No lack of variety exists in other nitrogenous components that have been reported in bacterial phospholipids; but these generally occur only in trace amounts. Aspartic and glutamic acids, glutamine, and necrosamine have been reported in *E. coli* phospholipids (*169*). No less than five amino acids have been reported in the various species of *Salmonella* studied (*174, 175, 179*); four amino acids in diphtheria bacteria (*178*), up to six in *Mycobacterium* (*172*), and seven in *Vibrio cholerae* excluding ornithine which is a major component (*173*).

Since the nitrogenous constituents of bacterial phospholipids already set them apart from the phospholipids of plants and animals, it is not surprising that they also differ with respect to the distribution of inositol and carbohydrate. Of the genera studied, only *Mycobacterium* consistently yields inositol on hydrolysis (*177, 180, 181*). It is not clear exactly what the structure of the inositide is; however, De Sütö-Nagy and Anderson (*182*) have isolated from the hydrolyzate of *M. tuberculosis* phospholipids a mannose-glyceryl diphosphate and a polysaccharide which yields on further hydrolysis two moles of mannose per mole of inositol. Phosphatidylinositol, phosphatidyl-D-mannose, phosphatidic acid, and a dimannoside of phosphatidylinositol have also been isolated (*181, 183*). The last two compounds contain saturated fatty acids only. Other bacterial phospholipids, those from *S. ballerup* (*174*) and *S. paratyphi* (*175*) have been reported to contain no carbohydrate or inositol although galactose phosphate has been isolated from *S. typhi* (*171*). A polysaccharide is associated with the phospholipids of *Lactobacillus acidophilus* which on further hydrolysis yields galactose, glucose, and fructose (*168*).

The isolation of glycerol phosphate from hydrolyzates of most bacterial lipids suggests that the classic glycerophosphatide structure may be present. An interesting exception to this is the report of Chargaff (*184*) of the complete absence of glycerol from *Corynebacterium diphtheriae* lipids. This was confirmed by Takahashi (*185*), who found dihydroxyacetone instead. The reported isolation of a compound resembling the cardiolipin of heart from this organism is directly contrary to the evidence for the absence of glycerol (*186*).

The fatty acids of bacteria are distinguished by the presence of branched chain, hydroxy- and cyclic-type structures which are often found in only one species, for example, mycolipenic acid in *M. tuberculosis* (*187*). Not all these acids are found in the phospholipids, nor is any one known to be confined to the phospholipids alone. Other acids, although found only in bacteria, are more widely distributed. Phytomonic acid found in the phospholipids of *Phytomonas tumefaciens* has been identified by Hofmann and Tausig (*188*) with lactobacillic acid which is found in *Lactobacillus acidophilus*, *L. arabinosus*, and *L. casei*.

It is of interest, however, that it is not found in the phospholipids of these lactobacilli (*189*). Normal straight-chain fatty acids both saturated and unsaturated are also found in bacterial phospholipids. The exact nature of these varies considerably with the conditions of growth; but in general, they are more saturated than fatty acids from phosphatides of plants and animals.

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CHAPTER 6

The Metabolism of Phospholipids

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In Chapter 5 it has been well documented that the phospholipids are ubiquitous in living things. This indicates that the enzymes responsible for their synthesis must also be widely distributed. All plants, for example, can be successfully grown in culture media devoid of phospholipids, which shows that they must possess adequate metabolic systems for synthesizing these compounds. This is also true with the higher animals such as the mammals, which can be successfully reared on phospholipid-free diets, and it shows that they can form all the new phospholipid material required for growth and development. This synthetic capacity must exist even before birth, as the transfer of intact plasma phospholipids through the placenta is minimal (*1*).

When the adult mammal is injected with labeled phosphate, acetate, choline, or ethanolamine there is a comparatively rapid incorporation

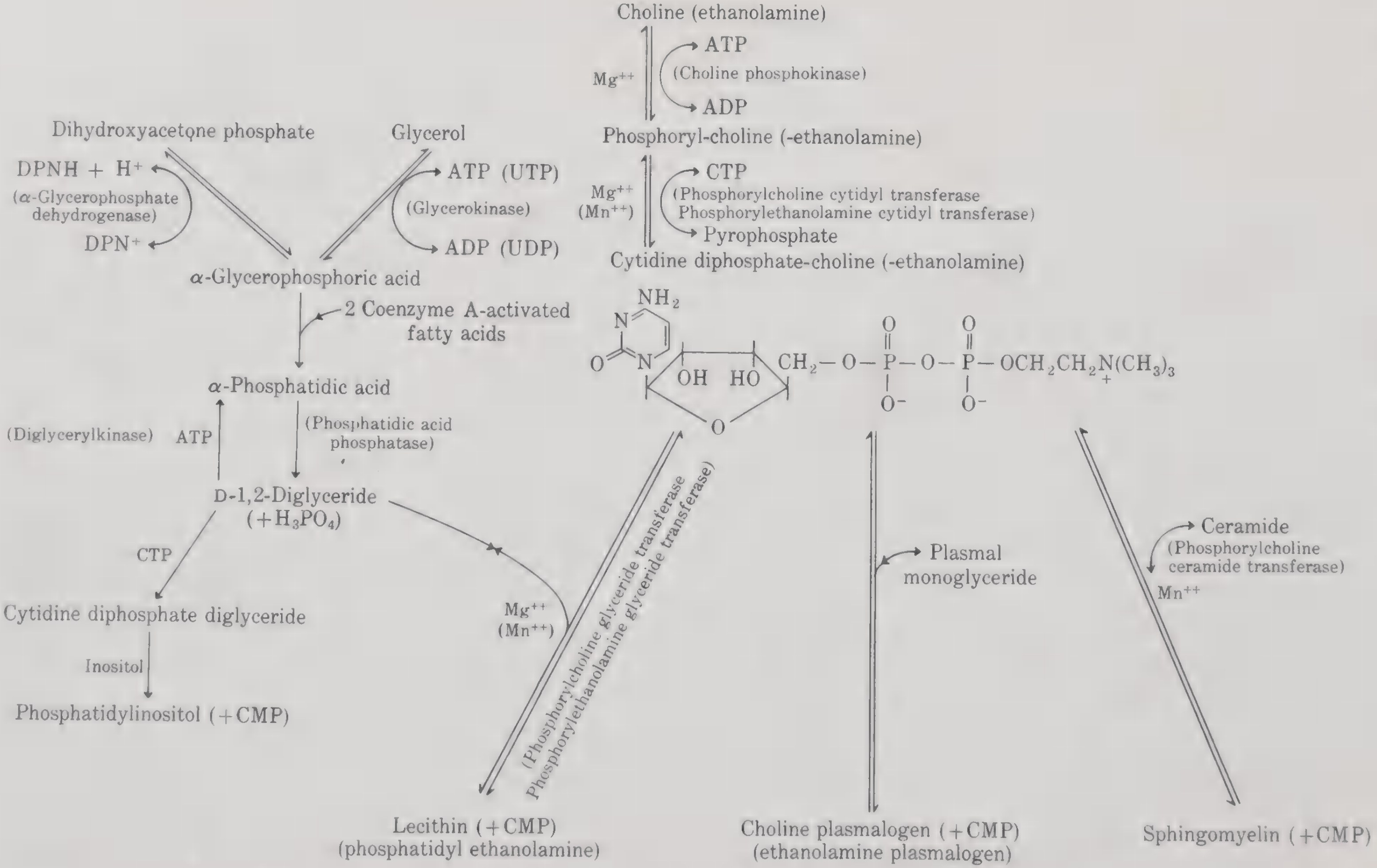
Choline (ethanolamine)
Mg++
ATP
(Choline phosphokinase)
ADP
Phosphoryl-choline (-ethanolamine)
Mg++
(Mn++)
CTP
(Phosphorylcholine cytidyl transferase
Phosphorylethanolamine cytidyl transferase)
Pyrophosphate
Cytidine diphosphate-choline (-ethanolamine)
Dihydroxyacetone phosphate
DPNH + H+
(α-Glycerophosphate dehydrogenase)
DPN+
Glycerol
ATP (UTP)
(Glycerokinase)
ADP (UDP)
α-Glycerophosphoric acid
2 Coenzyme A-activated fatty acids
α-Phosphatidic acid
(Diglycerylkinase)
ATP
(Phosphatidic acid phosphatase)
D-1,2-Diglyceride
(+H3PO4)
CTP
Cytidine diphosphate diglyceride
Inositol
Phosphatidylinositol (+CMP)
Mg++
(Mn++)
(Phosphorylcholine glyceride transferase
Phosphorylethanolamine glyceride transferase)
Plasmal monoglyceride
Ceramide
(Phosphorylcholine ceramide transferase)
Mn++
Lecithin (+CMP)
(phosphatidyl ethanolamine)
Choline plasmalogen (+CMP)
(ethanolamine plasmalogen)
Sphingomyelin (+CMP)

FIG. 1. Enzyme systems that can synthesize phospholipids *in vitro* and possibly *in vivo*.

of the label into the various tissue phospholipids; certain tissues, such as liver and kidney, are especially active in this respect. As no further new phospholipid is required for net growth, a rapid turnover of tissue phospholipids must be occurring to maintain physiological function. These isotopic incorporation experiments with adult animals not only indicate that there are active systems for synthesizing phospholipids in the animals, but also suggest by implication that catabolic systems must also be present in order to maintain a concentration equilibrium.

I. The Biochemistry of Phospholipid Synthesis

The enzymatic steps in the biosynthesis of phospholipids have been elucidated only comparatively recently, largely by the use of isotopically labeled intermediaries. In a notable contribution to the field, Kennedy and his co-workers have shown that the cytidine nucleotides participate as specific coenzymes. While it is apparent that this cytidine nucleotide-catalyzed type of synthesis must be a major source of the phospholipids that occur in living things, it remains to be seen whether any of the phospholipids are synthesized by other metabolic pathways e.g., via lysophospholipid (*2a*).

The chemistry of the biosynthesis of lecithin, phosphatidyl ethanolamine, plasmalogen, and sphingomyelin are conveniently dealt with together, as the pathways involved show many similarities. It must be emphasized, however, that although analogous chemical reactions occur for each lipid, the enzymes involved are usually quite different.

The reactions involved are shown in Fig. 1.

A. BIOSYNTHESIS OF CYTIDINE DIPHOSPHATE-"BASE" INTERMEDIATE

For each lipid the starting point of the synthesis is the phosphorylation of choline or ethanolamine by adenosine triphosphate (ATP) catalyzed by an enzyme which has been called choline phosphokinase (*3*). The enzyme has been purified 25-fold with an autolyzate of brewers' yeast as a starting material. It requires Mg^{2+} for its activity, and it is able to catalyze the phosphorylation both of choline and ethanolamine, but not serine.

The phosphorylcholine and phosphorylethanolamine formed in these reactions can react with cytidine triphosphate, giving cytidine diphosphate choline or cytidine diphosphate ethanolamine, respectively, and inorganic pyrophosphate. The catalysis of this freely reversible step is brought about by two separate enzymes, phosphorylcholine cytidyl transferase and phosphorylethanolamine cytidyl transferase (*6*). These are widely distributed in nature (Table I). The phosphorylcholine cytidyl transferase is an "insoluble" enzyme. It is completely specific for

TABLE I

REPORTED DISTRIBUTION OF THE ENZYMES CONCERNED WITH PHOSPHOLIPID SYNTHESIS[a]

Enzyme	Mammalian tissues					Yeasts	Higher plants	References
	Liver	Kidney	Brain	Heart	Other tissues			
Choline phosphokinase	Calf, rabbit, rat, pig	Rabbit, pig	Calf, rabbit rat	—	Intestinal mucosa of calf and rabbit	Brewers'	—	(*3*, *5*)
Phosphorylcholine cytidyl transferase	Rat, pig, guinea pig, chicken	Rat	Rat, calf	Rat	Rat seminal vesicles	Several strains	Carrot root	(*6–9*)
Phosphorylethanolamine cytidyl transferase	Rat, pig, guinea pig	Rat	Rat	Rat	—	Several strains	Carrot root	(*6*)
Phosphorylcholine glyceride transferase	Rat, chicken	—	Rat	—	Rat sciatic nerve, seminal vesicles	Brewers'	—	(*6*, *8–11*)
Phosphatidic acid phosphatase	Rat, chicken	Rat	Rat, guinea pig	Rat	Rat skeletal muscle	—	—	(*12*, *13*)
Glycerokinase	Rat, hen, pigeon	Rat	—	—	—	Bakers' and wild yeast grown on glycerol	—	(*14*, *15*)
Phosphatidic acid synthetase	Guinea pig	—	Guinea pig, rat	—	—	—	—	(*8*, *13*, *16*, *17*)
Phosphorylcholine ceramide transferase	Chicken, rat, guinea pig, pig	Rat	—	—	Rat spleen	—	—	(*18*)

[a] Such enzymes must occur far more widely than is indicated here.

cytidine triphosphate and it requires a divalent ion such as Mg^{2+} or Mn^{2+} for full activity.

B. Lecithin and Phosphatidylethanolamine Biosynthesis

The synthesis of lecithin and phosphatidylethanolamine from these cytidine-containing intermediaries (cytidine diphosphate choline and cytidine diphosphate ethanolamine) is brought about by the transfer of the phosphorylated base unit to a D-1,2-diglyceride with the release of cytidine monophosphate. Again two different enzymes are responsible [phosphorylcholine glyceride transferase and phosphorylethanolamine glyceride transferase (*6*)]. The reactions are again reversible (*10*); they require Mg^{2+} or Mn^{2+} but are completely inhibited by low concentrations of Ca^{2+} (10^{-3} M). The presence of a surface active agent, e.g., bile salts, digitonin, or Tween 20, stimulates the lecithin synthesis ten to twentyfold probably by removing diglyceride from the surface of the enzyme. Phosphorylcholine glyceride transferase is highly specific for cytidine diphosphate choline, and analogous compounds containing uridine, guanine, and adenosine are quite ineffective in the system. It appears, however, that deoxycytidine diphosphate choline and deoxycytidine diphosphate ethanolamine can also be used for the synthesis of lecithin or phosphatidylethanolamine (*19*).

C. Formation of D-1,2-Diglyceride and Phosphatidic Acid

It now seems likely that the formation of the D-1,2-diglyceride required as an acceptor for the phosphorylcholine or phosphorylethanolamine units is produced by the dephosphorylation of phosphatidic acid. The phosphatase that brings about this hydrolysis has a specific action on phosphatidic acid (*12*). It appears to be bound in particulate matter and is inhibited by Mg^{2+} and other divalent ions, which probably form insoluble salts with the substrate. The phosphatidic acid required for the formation of D-1,2-diglyceride is itself formed from L-α-glycerophosphoric acid. The glycerophosphoric acid required can be formed either by the enzyme glycerophosphate dehydrogenase utilizing the glycolysis intermediary dihydroxyacetone phosphate as precursor or by the direct phosphorylation of glycerol by ATP or uridine triphosphate (UTP) catalyzed by the enzyme glycerokinase (*14, 15*). This latter enzyme has now been crystallized; it requires Mg^{2+} or Mn^{2+} for activity. The formation of phosphatidic acid from the glycerophosphate is brought about by an enzyme system that is present in liver particles and catalyzes the transfer of two fatty acids from coenzyme A (*16*). At present the mechanism of the acylation is not known, and it is possible that there is a stepwise addition of the two acyl groups on the vacant hydroxyl groups of the glycerol. In brain tissue, phosphatidic acid can be synthesized both by the acylation of glycerophosphate and also by the direct phosphorylation of a diglyceride by ATP (*13*). It is clear from Fig. 1, however, that this latter reaction cannot play a part in the net synthesis of lecithin or phosphatidylethanolamine.

D. SPHINGOMYELIN BIOSYNTHESIS

Sphingomyelin can be synthesized by chicken liver preparations through an enzyme that catalyzes the transfer of phosphorylcholine from cytidine diphosphate choline to a ceramide with the elimination of cytidine monophosphate (*18*). This reaction brought about by the enzyme phosphorylcholine ceramide transferase, is analogous to the transfer of phosphorylcholine to diglyceride in the synthesis of lecithin. The enzyme responsible differs, however, in that it requires Mn^{2+} for maximum activity.

The best ceramide acceptor is *N*-acetylsphingosine, and activity of the transferase decreases as the chain length of the acyl group is increased. However, in the presence of an emulsifying agent (Tween 20) the activity increases up to a maximum with eight carbon atoms in the chain length: with chain lengths longer than twelve, activity is found only if unsaturated bonds are present to assist the emulsification (e.g., *N*-oleyl and *N*-linoleyl sphingosine). A curious property of the enzyme system is that it appears to be specific for ceramides with the *threo* configuration whereas the ceramides present in sphingomyelin isolated from tissues have the *erythro* configuration. The sphingomyelin which has been synthesized *in vitro* has the *threo* configuration, so clearly there is some difference between the synthesis of sphingomyelin *in vitro* and *in vivo*. Possibly in tissues an enzyme may be present that can interconvert *threo*- and *erythro*-sphingomyelin.

E. PLASMALOGEN BIOSYNTHESIS

Although the mechanism of the biosynthesis of choline and ethanolamine plasmalogen has not yet been elucidated, there is good evidence that it is analogous to that of lecithin and phosphatidylethanolamine. The synthesis is stimulated by the presence of cytidine triphosphate and glycerophosphate (*20*), and recently Kiyasu and Kennedy (*20a*) have found that a plasmal monoglyceride can act as an acceptor for the phosphorylcholine unit from cytidine diphosphate choline, using an enzyme obtained from rat liver. The plasmal monoglyceride used in these experiments was prepared by hydrolysis of a plasmalogen-rich lecithin fraction with *Clostridium perfringens* (*welchii*) phospholipase C to remove the phosphorylcholine moiety, followed by the separation of the diglyceride and plasmal monoglyceride on a silicic acid column. It is not known whether this is the means by which naturally occurring plasmalogens are formed or how such a plasmal monoglyceride acceptor could be synthesized in nature.

F. BIOSYNTHESIS OF PHOSPHATIDYLINOSITOL AND POLYGLYCEROL PHOSPHOLIPIDS

Recently it has been shown that cytidine coenzymes participate in the *in vitro* synthesis of inositol phospholipids (*21–23*). The mechanism of the synthesis of phosphatidylinositol is, however, markedly different from that of lecithin or phosphatidylethanolamine in that the diglyceride molecule is first converted into the compound cytidine diphosphate diglyceride (Fig. 1). This is brought about by an enzyme found in guinea pig liver and kidney. At present the source of the diglyceride is not known, although it is possible that it is formed from phosphatidic acid as in the biosynthesis of the other phosphoglycerides. This cytidine diphosphate diglyceride in the presence of a liver enzyme can react directly with free inositol to form phosphatidylinositol and free cytidine monophosphate.

It is also likely that cytidine diphosphate diglyceride is an intermediary in the biosynthesis of polyglycerol phospholipids such as phosphatidylglycerol or cardiolipin. It is known, for example, that an enzymatically catalyzed reaction can occur between cytidine diphosphate diglyceride and L-α-glycerophosphate (Kennedy, unpublished) which produces a diglyceride phosphorylglycerophosphate.

G. DISTRIBUTION OF ENZYME SYSTEMS RESPONSIBLE FOR PHOSPHOLIPID BIOSYNTHESIS

Table I shows the reported distribution in nature of the enzymes that participate in phospholipid synthesis. It must be emphasized, however, that in the references quoted it was not the primary objective of the work to ascertain the comparative distribution of such enzymes, and clearly they must occur very much more widely if not universally in all the living cells that synthesize phospholipids. However, it is known that one of the synthetic enzymes, namely phosphorylcholine cytidyl transferase, is absent from many bacteria (*7*). This may explain why there is generally an absence of lecithin in bacterial phospholipids.

In mammals, it is becoming increasingly obvious from isotopic studies on slices of tissue from different organs that each tissue possesses the requisite enzymes for the complete *de novo* synthesis of phospholipid molecules. If such slices are incubated in the presence of labeled precursors of phospholipids, such as glycerol, orthophosphate, or acetate, it is found that, provided suitable metabolic conditions pertain, the individual phospholipids isolated from the slice become radioactive. This applies even to such organs as the thyroid, pituitary, or pancreas glands (*24–26*). The transport of freshly synthesized intact phospholipid molecules from tissue to tissue via the plasma is thought to be small. If, for example, labeled acetate or phosphate is injected into animals in which the synthesis of the plasma phospholipids has been prevented by hepatectomy, the incorporation of isotope into the phospholipids of other organs is more or less normal (*26a,b*). We are left, therefore, with a picture of each cellular tissue acting as an independent synthetic unit for the new phospholipids it requires for growth and replacement.

The liver appears to be the sole site of synthesis of plasma phospholipids in the fasting dog and rat. This is shown by removing the liver from these animals and injecting a labeled phospholipid precursor, e.g., phosphate, when it is found that negligible incorporation into the plasma phospholipids occurs compared with that in the normal animal (*26a,b, 27*). In the nonfasting rat however some plasma phospholipid can be synthesized in the intestine, especially when the animal is digesting a high fat-content meal. In the fasting rabbit (*28*) and chicken (*29*) the liver is again the major site for plasma phospholipid synthesis, but some plasma phospholipids can be formed in extrahepatic tissues. Although there is no evidence that significant amounts of plasma phospholipid can be formed by the bone marrow (*30*), recent work indicates that in man some of the phospholipids in the plasma β-lipoprotein can be synthesized in the blood cells (*31*), especially the leukocytes.

II. Enzyme Systems That Degrade Phospholipids

Enzymes that degrade phospholipids have been studied and characterized for much longer than those responsible for their synthesis. The most extensively studied substrate has been lecithin, hence in the older literature the enzymes are usually called lecithinases. More recently it has been realized that these enzymes attack other substrates than lecithin, and the term phospholipase has been widely accepted. The phospholipases are usually classified from the ester bond of lecithin which they hydrolyze. A difficulty arises in that sharp controversy still exists as to whether the α or β fatty acid is removed from lecithin by phospholipase A (*32*). Furthermore, it is now known that under certain circumstances, phospholipase B can remove both fatty acids (*33*). Consequently, for the purpose of this article, phospholipase A is defined as the enzyme that removes a single fatty acid from lecithin, forming lysolecithin; phospholipase B, as the enzyme that hydrolyzes the fatty

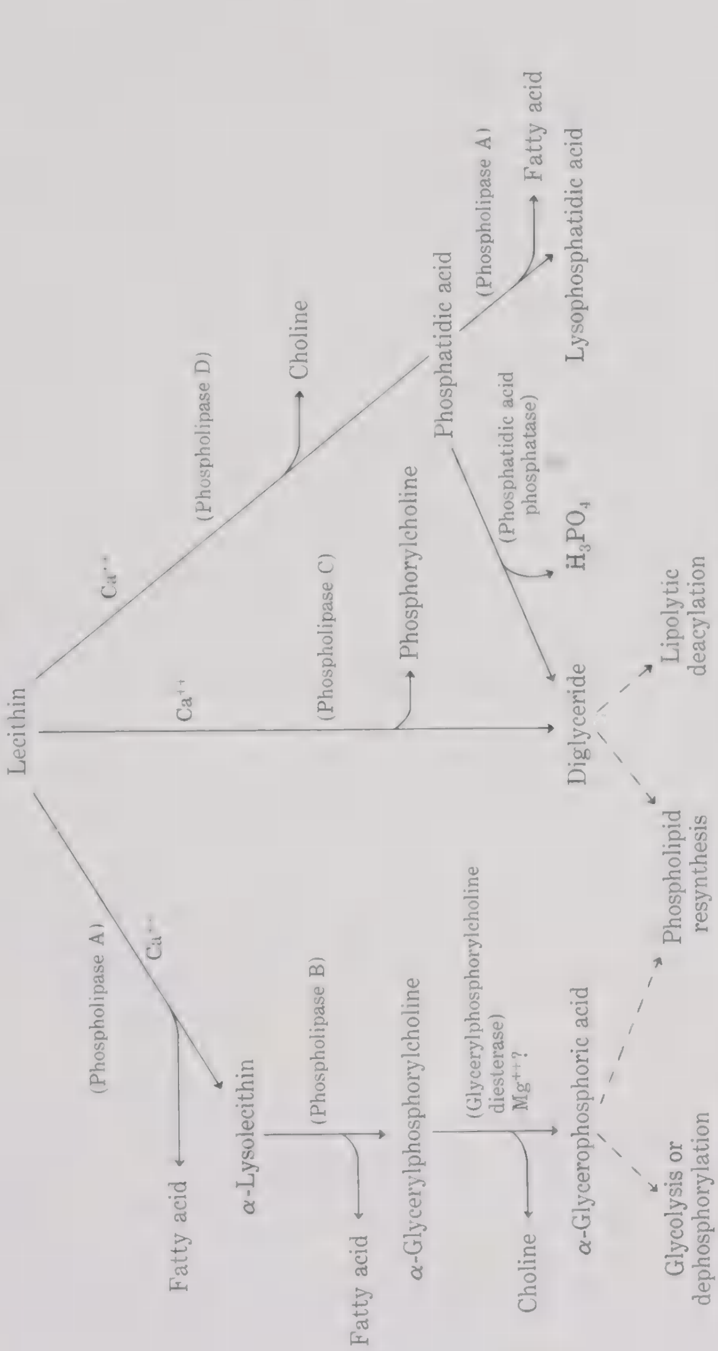

FIG. 2. Enzymes that can catalyze the hydrolytic degradation of lecithin.

acyl ester bond of lysolecithin as its preferred substrate; phospholipase C, as the enzyme that catalyzes the hydrolysis of lecithin at the ester bond between the diglyceride and phosphorylcholine moieties; and phospholipase D, between the phosphatidic acid and choline moieties. Some authorities classify the latter two enzymes in the reverse order, i.e., phospholipase C as the enzyme that hydrolyzes choline from lecithin, but the present system is preferred as it is more systematic and is in accord with the order in which the enzymes were discovered.

The metabolic steps catalyzed by these phospholipases and the associated enzymes responsible for the further degradation of the hydrolysis products are shown diagrammatically in Fig. 2.

A. PHOSPHOLIPASE A

1. *Properties*

Phospholipase A catalyzes the hydrolysis of lecithin with the formation of lysolecithin and a single fatty acid. Until recently it was thought that the α-fatty acid was the one removed by snake venom phospholipase A, but recent evidence suggests that it may be the β fatty acid (*32*). The enzyme is quite specific for lecithin with the L-configuration and α-structure (*34*). With lecithin isolated from natural sources, e.g., ovolecithin, the enzyme preferentially removes unsaturated fatty acids, which could merely reflect a positional localization of these on the lecithin molecule. However, lecithins containing fully saturated fatty acids are readily attacked (*35*).

As well as lecithin, phospholipase A will also attack phosphatidylethanolamine (*34, 34a*). There is also some evidence that phosphatidylserine, phosphatidic acid, ethanolamine plasmalogen, and choline plasmalogen are also attacked, although phosphatidylinositol is not hydrolyzed (*34, 34a, 35*).

The phospholipase A enzymes of venoms and the pancreas are unusual in two respects. First, they have a remarkable resistance toward heat, especially below pH 7, and the venom enzyme will even stand boiling at pH 5.9 for some minutes (*36*). Secondly, their catalytic activity is greatly stimulated in the presence of ether. The enzyme appears in fact to form an ether-soluble enzyme substrate complex so that it readily works in wet ethyl ether containing dissolved lecithin substrate (*35, 37*). Under these conditions the lysolecithin formed is precipitated from the ether, carrying down the enzyme with it, but the enzyme continues to catalyze the hydrolysis of the lecithin substrate until breakdown is complete. Bacterial phospholipase A from *Serratia plymuthica* is apparently not active in ether solution (38), but this enzyme has not yet been prepared in a soluble form.

There is almost universal agreement that phospholipase A can be stimulated by the presence of calcium ions, although there is also evidence that excess calcium can be inhibitory. The pH optima of the enzymes from all sources lie between 6.8 and 7.7, a comparatively small variation compared with the wide divergence seen with phospholipase B.

2. *Distribution*

Phospholipase A is perhaps best known as a constituent of the venom of snakes and partly accounts for the well-known hemolytic properties of the poisons—the lysolecithin formed by its action causing hemolysis of erythrocytes. It is also present in the poisons and venoms of many other animals including, e.g., wasps, bees, and scorpions. Table II shows the species whose venoms have been characterized as containing phospholipase A. The amount of enzyme present in the venoms varies from species to species, but in all cases it is not the only toxin present and often not even an important one. In some snake venoms it is present in large amounts and can be crystallized by comparatively simple procedures (Table II).

In mammalian tissues, phospholipase A has been most extensively studied in the pancreas. As with snake venoms the enzyme shows considerable resistance to heat, and it has considerable solubility in aqueous ethanol solutions (*34a*, *54*, *55*). It appears to be present in the pancreas of most mammals, although there is little in the pancreatic juice of ruminants, e.g., sheep or ox. It occurs in the hepatopancreatic juice of *Helix pomatia* (*55a*). Phospholipase A has been characterized also in pig lung (*55*) and in rat intestinal mucosa (*56*). The particulate enzyme isolated from the latter source is activated by the addition of free fatty acids; it is unusual in that it is inhibited by calcium ions in low concentrations. A number of reports in the older literature (e.g., *57*) suggest that the enzyme may be present in other mammalian tissues such as liver, kidney, heart muscle, adrenals, but the enzyme was not clearly characterized as being of the A type.

An active phospholipase A is present in the bacterium *Serratia plymuthica*, especially when this is grown in a lecithin-enriched medium (*38*). This bacterial enzyme is thermolabile and Ca^{2+} is required for full activity. It is possible that ferrous ions also may be needed as a cofactor.

It is likely that a phospholipase A is present in ram spermatozoa

TABLE II

SOME SPECIES WHOSE VENOM HAS BEEN SHOWN TO CONTAIN PHOSPHOLIPASE A

Species	Common name	Reference
Snakes		
Family Elapidae		
Bungarus coeruleus	Indian krait	(*39*)
Bungarus fasciatus[a]	Banded krait	(*40*
Denisonia superba	Australian copperhead	(*36*)
Dendraspis viridis	Green mamba	(*39*)
Naja flava (nivea)	Yellow cobra, Cape cobra	(*41*, *42*)
Naja haje	Egyptian cobra	(*43*)
Naja naja	Indian cobra	(*34*)
Naja nigricollis	Black-collared cobra	(*39*, *43*, *44*)
Naja tripudians[a]	Cobra	(*40*)
Notechis scutatus	Black tiger	(*36*)
Pseudechis porphyriaceus	Black snake	(*36*)
Family Hydrophidae		
Enhydrina schistosa	Sea snake	(*45*)
Family Viperidae		
Bitis gabonica	Gaboon viper	(*39*)
Echis carinatus	Sandburrowing viper	(*39*)
Vipera ammodytes	European nose-horned viper	(*42*, *44*, *46*)
Vipera aspis	S.W. European viper	(*39*)
Vipera berus	Common European viper	(*44*)
Vipera elegans	Daboia	(*36*)
Vipera russelli	Russell's chain viper	(*39*)
Family Crotalidae		
Agkistrodon p. piscivorus	Cottonmouth moccasin	(*34*, *37*)
Bothrops alternatus	Urutu	(*47*)
Bothrops jararaca	Jararaca	(*39*, *48*)
Bothrops neuwiedi	Maximilian's viper	(*39*)
Crotalus adamanteus	Diamondback rattlesnake	(*34*)
Crotalus atrox	Western diamond rattlesnake	(*39*)
Crotalus crotaminicus	Brazilian rattlesnake	(*49*)
Crotalus terrificus[a]	Mexican rattlesnake	(*41*, *44*, *50*, *51*)
Arthropods		
Order Hymenoptera		
Apis mellifera	Honeybee	(*39*, *52*)
Bombus pratorum	Bumblebee	(*39*)
Vespa vulgaris	Yellow jacket wasp	(*39*)
Class Arachnida		
Buthus occitanus	Scorpion	(*39*)
Heterometrus maurus	Scorpion	(*39*)
Isometrus maculatus	Scorpion	(*39*)
Aranea diadema	Spider	(*39*)
Buthacus arenicola	Scorpion	(*53*)
Scorpio maurus	Scorpion	(*53*)

[a] Enzyme has been crystallized from the venom.

and that this is responsible for the liberation of a free fatty from the choline plasmalogen which they contain (*57a*). Evidence has also been obtained that a similar enzyme is present in sea urchin spermatozoa (*58*).

B. PHOSPHOLIPASE B

1. *Properties*

The preferred substrate of this enzyme is lysolecithin or lysophosphatidylethanolamine, which it attacks, liberating the esterified fatty acid and leaving glycerylphosphorylcholine or glycerylphosphorylethanolamine. Until recently it was believed that the enzyme could not hydrolyze diacylated phosphoglycerides, but partially purified preparations of the enzyme from *Penicillium notatum* can slowly hydrolyze lecithin in the presence of certain naturally occurring activators, e.g., phosphatidylinositol, polyglycerol phospholipid (*59*), which appear to function by introducing on the substrate surface a certain minimum concentration of negatively charged polar groups (*33, 60*). No separation of the enzymatic activity toward either substrate (lysolecithin or activated lecithin) could be obtained by electrophoresis of the enzyme under a variety of conditions. The mold enzyme preparation can also slowly attack phosphatidylinositol, liberating glycerylphosphorylinositol (*61*).

Phospholipase B differs from other phospholipases in that its activity toward lysolecithin is not stimulated by the addition of ether. This may reflect the fact that lysolecithin goes into colloidal solution in water in the form of micromicelles (*62*). The enzyme does not require calcium for full activity as do the A, C, and D phospholipases.

2. *Distribution and Comparison of Phospholipase B from Various Sources*

Phospholipase B is very widely distributed in nature, where it usually occurs in association with phospholipase A. Under such circumstances, lysolecithin hydrolysis always occurs at a much faster rate than that of lecithin, a device that probably protects living cells from the accumulation of toxic lysophosphoglycerides, which have a disrupting effect on cell membranes.

In mammalian tissues, phospholipase B occurs in the pancreas, from which it may be extracted and purified to a crystalline form (*63*). The enzyme has been studied also in rat liver (*64*) and in rat intestinal mucosa (*56*) and is present in rat kidney, ileum, testis, skeletal muscle, brain, heart, spleen, and whole blood as well as sheep liver and thyroid (*2, 64*) and pig lung (*55*). Recent evidence suggests that it may also be present in human plasma. The mammalian tissue enzyme has a pH optimum of 6.0–6.5; this is much higher than that of the phospholipase B of molds.

Phospholipase B has been prepared from fresh extracts of rice bran or *Aspergillus oryzae* (*65*); the mycelium of *Penicillium notatum* is also a rich source (*66*) as well as that of *Penicillium chrysogenum* (*67*). The enzyme from mold has been partially purified with ammonium sulfate (*64, 68*). It has a low pH optimum, which has been found with various preparations to vary between 3.3 and 4 (*59, 65, 66*).

In bacteria, phospholipase B has been characterized in *Serratia plymuthica;* it has a pH optimum of 6.0 and differs from the enzyme prepared from other sources in having remarkable thermal stability (*38*). It has been found also in *Mycobacterium avium,* the avian tubercule bacillus (*69*), and in a preparation of microorganisms from sheep rumen (*70*). The partially purified enzyme from the latter source, which could be derived from either protozoa or bacteria, has a pH optimum of 7.5, which is higher than the phospholipase B from other sources.

Phospholipase B does not occur in the poison of snakes, but with the venoms of the bee, hornet (*Vespa crabro*), and wasp, the lysolecithin formed by the action of phospholipase A is further broken down to glycerylphosphorylcholine (*65, 71, 72*).

C. Glycerylphosphorylcholine Diesterase

The glycerylphosphorylcholine and glycerylphosphorylethanolamine formed by the action of phospholipase B can be further metabolized by the enzyme glycerylphosphorylcholine diesterase, which catalyzes the hydrolysis of both these phosphodiesters, liberating the free base and glycerophosphoric acid. The enzyme has been characterized in mammalian tissues [rat liver, kidney, heart, brain, spleen, intestine, skeletal muscle, and blood cells (*73*) and in various parts of the nervous system in the rat, guinea pig, hen, human, rabbit, and dog (*74*)] and also in the bacterium *Serratia plymuthica* (*38*). The enzyme does not appear to be present in significant amounts in sheep liver (*73*) or in pancreas (*63*).

Soluble preparations of the liver enzyme require Mg^{2+} for full activity, although high concentrations are inhibitory (*73*). The bacterial enzyme is inhibited by Mg^{2+} and also by metal chelating agents such as Versene (*38*). The pH optimum of the rat liver enzyme is 7.5; of the bacterial enzyme, 9.0; and of rat brain homogenates, 9.5.

D. PHOSPHOLIPASE C

1. *Properties*

This phospholipase catalyzes the hydrolysis of lecithin, liberating a 1,2-diglyceride and phosphorylcholine. Both fully saturated and unsaturated lecithins are attacked (*75*), and also choline plasmalogen. Sphingomyelin is hydrolyzed with the formation of phosphorylcholine and a ceramide, but phosphatidylserine, phosphatidylethanolamine, and lysolecithin are not usually attacked (*75, 75a*) although there is some evidence that they can be hydrolyzed when they are presented to the enzyme in a mixed phospholipid substrate (*75b*).

The activity of the enzyme under normal conditions of assay requires the presence of calcium as an activator (*76*). To a lesser extent magnesium, maganese, cobalt, and zinc can activate the system, but cuprous, strontium, cadmium, aluminum, ferrous, ferric, and barium ions are inhibitory (*77*). Phospholipase C is similar to the A and D enzymes in showing considerable resistance to heat and also by its ability to act catalytically in wet ether solution of its substrate (*75*). The enzyme has been partially purified by ammonium sulfate and nucleic acid precipitation (*78*) and by methanol solvent fractionation (*79*).

2. *Distribution*

Phospholipase C appears to be largely confined to the plant kingdom although reports, as yet unconfirmed, have appeared of its presence in fetal pig cartilage (*80*) dog brain, rabbit pancreas, ox kidneys (*81*), human semen (*82*), and the venom of the snake *Bothrops alternatus* (*47*).

In bacteria, phospholipase C is identical with the α-toxin of *Clostridium perfringens* (*welchii*) and also that of *C. bifermentans* (*83*). It is also found in *C. hemolyticum* (*84*) and *C. oedematiens* (*novyi*) (*85*). Although the enzymes from these species all catalyze the identical chemical reaction, they are not by any means identical proteins. For example, in *C. oedematiens* filtrates two immunologically distinct lecithinases are present; these probably represent the γ- and β-toxins. The phospholipase in *C. hemolyticum* is apparently identical antigenically with *C. oedematiens* β-toxin, but not with the γ-toxin or the α-toxin of *C. perfringens*. The *C. bifermentans* lecithinase is inhibited by *C. perfringins* antitoxin; this suggests similarity of structure, but nevertheless, the two lecithinases show appreciable differences in their biochemical properties [e.g., the pH optimum of the *C. perfringens* enzyme, 8.3, is higher than that of the *C. bifermentans*, 6.2 (*83*)] and differences in their toxicity (*86*). This indicates therefore that the enzymatic active centers are not identical even though the antigenic groups on their molecules may be similar.

The phospholipase in *Bacillus cereus* and *B. cereus* var. *mycoides* has been characterized as of the C type. In both these organisms the phospholipases are immunologically related, but they are not so related to *C. perfringens* phospholipase C (*87*). The phospholipase C of *B. cereus* is probably the "thromboplastinase" isolated from strains of this organism, which greatly reduces the thromboplastic activity of a tissue suspension (*75b*).

Recent evidence suggests that phospholipase C can occur in higher plants. Barley mitochondria have been shown to attack lecithin with the liberation of phosphorylcholine (*88*); this may explain the high concentration of phosphorylcholine found in barley seedlings (*89*). Kates (*90*) has found that spinach chloroplasts can hydrolyze lecithin, liberating phosphorylcholine.

E. Phospholipase D: Properties and Distribution

Phospholipase D, which catalyzes the hydrolysis of choline from lecithin, leaving a phosphatidic acid, appears to be confined to the higher plants. The enzyme was first demonstrated in the carrot and cabbage (*91*), and it is probably responsible for the phosphatidic acid which older work had suggested was a constituent of cabbage leaves (*92*). The enzyme from cabbage acts readily upon phosphatidylethanolamine and more slowly upon lysolecithin (*93, 94*). It appears to exist in the plant cells as an insoluble enzyme associated with the plastids (*95*), but a soluble form can be readily demonstrated in the cabbage (*93*) as well as in the carrot (*96*) and in cottonseed (*94*).

The soluble enzyme requires the presence of calcium for maximal activity (*93, 96*). Both the insoluble plastid enzyme and the soluble enzyme of the carrot and cabbage are greatly stimulated by the addition of ether to the reaction mixture (*93, 95*). On the other hand, the phospholipase D of cottonseed does not appear to be activated by this solvent (*94*). The plastid enzyme is activated also by other solvents, e.g., linear aliphatic ethers, ketones, and esters as well as anionic detergents (*97*). Solvents that stimulate the reaction have been shown to produce coalescence between lecithin micelles and chloroplasts (*98*). The partially purified soluble enzyme from cabbage is stimulated by water-soluble phosphodiesters (*93*) as well as by phosphatidylinositol (*99*). These observations suggest that the reaction between the soluble enzyme and its substrate may be controlled by electrostatic forces analogous to those of the *Penicillium notatum* phospholipase B-lecithin interaction (*33*, *60*).

The pH optimum of the enzyme lies between 4.8 and 6 and seems to depend less on the source than on the mode of determination of activity. In general its resistance to heat appears to be less than that of phospholipases A and C (*94–96*).

Two of the richest sources of phospholipase D are the Savoy cabbage and brussels sprouts, and in these it appears to be selectively located in the inner leaves (*93*). Smaller activities have also been described in the turnip, potato, beet root, and pea plants (*93*) as well as in rutabago, spinach, green stringbean, sweet corn, and onion (*96*). In cereals it is found in wheat, barley, and oats (*100*), and it has also been described in the latex of *Hevea brasiliensis* (*101*).

F. OTHER ENZYME SYSTEMS THAT DEGRADE PHOSPHOLIPIDS

An enzyme has been detected in ox pancreas (*61*) and rat liver (*102*) which catalyzes the hydrolysis of monophosphoinositide to phosphorylinositol and a diglyceride (monophosphoinositidase C). Impure preparations of this enzyme require Ca^{2+} ions for full activity, and they are inactive toward lecithin or phosphatidylserine, although lysolecithin is attacked. Diphosphoinositide, the complex inositol-containing lipid of the nervous system, can be broken down rapidly by homogenates from guinea pig brain, liver, kidney, and spleen, yielding among other products phosphorylinositol and free inositol (*103*, *104*).

In mammalian tissues, a number of enzymes have been described which hydrolyze sphingomyelin (cf., e.g., *105*), but none of these have been completely characterized. There is some evidence, however, that spleen extracts can hydrolyze sphingomyelin with the formation of a ceramide and phosphorylcholine (*106*).

A number of workers have advanced claims that tissues such as liver and spleen possess phospholipid dehydrogenases (cf., e.g., *107*, *108*), as it was observed that the reduction of indophenol dyes was increased by the addition of lecithin or sphingomyelin. Such an oxidation, however, cannot be properly understood until the enzymatic substrate and reaction products have been fully characterized.

III. The Pathways of Phospholipid Metabolism *in Vivo*

The characterization of enzymes *in vitro* gives an invaluable guide as to the mechanism by which metabolism occurs in the living cell, but it by no means proves that the same pathway is operative *in vivo*. In mammalian tissues, however, there is certain additional evidence which suggests that the synthesis of phosphoglyceride *in vivo* is via the same cytidine coenzyme pathway indicated by the characterization of enzymes *in vitro* and also that one pathway for the catabolism of lecithin and phosphatidylethanolamine involves the formation of glycerylphosphorylbase intermediaries. Thus tissues have been shown to contain all the necessary intermediaries for both the synthesis and breakdown via these pathways, e.g., phosphorylcholine and phosphorylethanolamine, cytidine diphosphate choline and cytidine diphosphate ethanolamine, phosphatidic acid, glycerylphosphorylcholine and glycerylphosphorylethanolamine. More direct evidence has, however, been obtained by examining the phosphoglycerides and these intermediaries for precursor-product relationships after the injection of radioactive isotopes. In this way it has been shown that the glycerylphosphorylcholine and glycerylphosphorylethanolamine in rat liver are on the pathway of lecithin and phosphatidylethanolamine degradation and that the phosphorylcholine could not have possibly been formed by catabolism (*109, 110*). Similar results have been found for the sheep thyroid gland (*24*).

In more extensive isotopic studies on rat brain, evidence was obtained that the synthetic pathway for lecithin P *in vivo* is via phosphorylcholine and cytidine diphosphate choline and that the degradation is via glycerylphosphorylcholine (*111*).

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CHAPTER 7

Natural Monosaccharides and Oligosaccharides: Their Structures and Occurrence

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Foreword

The aim of this chapter is to present to those interested in the comparative aspects of biochemical knowledge: (i) a general account of the structures of the monosaccharides, both as interrelated free chemical individuals and as units out of which more complex natural molecules are wholly or partly constructed; and (ii) a report on the natural distribution of the monosaccharides, both in the free state and in chemical combinations.

The writer makes no claim to infallibility; much important and interesting information must undoubtedly have been overlooked. Since the advent of methods of isolation and separation of sugars based on column and partition chromatography and on electrophoresis, the flow of publications relating to carbohydrate biochemistry has become magnified from the placid stream of the 1930's to the tumultuous torrent of the 1950's. When in the early 1930's the writer was a student of carbohydrate chemistry, natural monosaccharides could be roughly numbered as about twenty; in the early months of 1960 the reader will see how this family has increased manyfold. In his early days the writer was told by some of his seniors—working in other subjects, however—that he would be ill-advised to pursue the study of carbohydrates "as the field had now been fully worked out"!

When endeavoring to plan this chapter two lines of approach presented themselves. The one was to treat the subject from the basis of occurrence of sugars according to phyla, genera, etc.; the other was to base divisions and subdivisions on the sugars themselves. It very soon became apparent that the second alternative was the only one possible since enormous lacunae exist in our knowledge of the presence of chemical substances in even closely related species. This field presents enormous opportunities for work with animals, plants, and microorganisms, especially in relation to genetics. In connection with the latter approach, the human familial conditions of L-xylulosuria, D-galactosemia, and the glycogen storage diseases may be recalled.

The physiological roles of many of the natural simple sugars and their derivatives are quite unknown and present the biologist with another vista of unexplored science. The field can hardly be said, nowadays, to have been worked out.

The reader may object that certain substances obviously derived from carbohydrates are not included in this chapter. The sialic acids, muramic acid, and sugar oxo acids are conspicuous examples. The writer humbly pleads that, although asked by the Editors to cast his net as wide as possible, he just had to draw the line somewhere. Without the aid provided by the Chemical Society's publication, *Current Chemical Papers* the whole task would have proved well-nigh impossible. The literature has been surveyed to December, 1960.

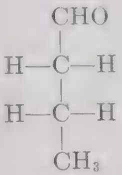
I. The Monosaccharides

The monosaccharides are compounds, each of which, as a rule, can exist in several different interconvertible, structural arrangements. The simplest of all these structures is the so-called *open-chain* form where the sugar has the structure of an aldehyde, or a ketone, derived from a polyhydroxybutane, -pentane, -hexane, -heptane, or higher molecule in which all or the majority of the noncarbonyl carbon atoms are hydroxylated. In all but rare instances the parent hydrocarbon belongs to the unbranched or *normal* series. As will become evident later, the biologically important structures of the monosaccharides, and of their derivatives, are rarely if ever the "aldo" or "keto" forms. Nevertheless a survey of these simple open-chain structures, with the help of their "projection formulas" introduced by Emil Fischer (*1*) serves best to illustrate the isomerism and stereochemical relationships existing between the different members of the family; certain special features anent the nomenclature of carbohydrates (2) will be considered at the same time.

A. THE D- AND L-FAMILIES OF MONOSACCHARIDES

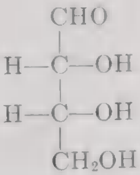
1. *Tetroses*

The simplest true monosaccharides that can exist in structural forms other than that of the simple open-chain polyhydroxy aldehydes are the four aldotetroses (II–V), so-called because they are derived from the four-carbon chain of the aldehyde butanal (I).



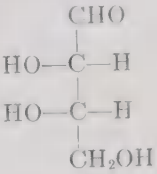
$$\begin{array}{c} CHO \\ | \\ H-C-H \\ | \\ H-C-H \\ | \\ CH_3 \end{array}$$

(I)



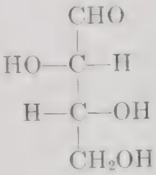
$$\begin{array}{c} CHO \\ | \\ H-C-OH \\ | \\ H-C-OH \\ | \\ CH_2OH \end{array}$$

(II)



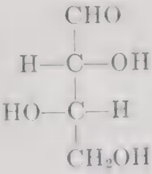
$$\begin{array}{c} CHO \\ | \\ HO-C-H \\ | \\ HO-C-H \\ | \\ CH_2OH \end{array}$$

(III)



$$\begin{array}{c} CHO \\ | \\ HO-C-H \\ | \\ H-C-OH \\ | \\ CH_2OH \end{array}$$

(IV)

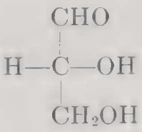


$$\begin{array}{c} CHO \\ | \\ H-C-OH \\ | \\ HO-C-H \\ | \\ CH_2OH \end{array}$$

(V)

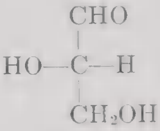
Considering the projection formulas (II–V) it will be seen that in all these fully hydroxylated but isomeric butanals, carbon atoms 2 and 3 (C-2 and C-3) are centers of asymmetry and that two separate pairs of optical enantiomorphs are represented by (II) with (III) and by

(IV) with (V). It can be shown experimentally that the configuration of asymmetric centers at C-3 of two of the four compounds (II and IV) is identical with that of D-(+)-glyceraldehyde (VI) and that the configuration at C-3 of the other two (III and V) is identical with that of L-(−)-glyceraldehyde (VII). The stereochemical family to which a monosaccharide belongs (*3*) is determined by the configuration of the asymmetric center at that carbon atom which is distinguished by the largest number in the chain, that is, the asymmetric center farthest from the carbonyl group in the open-chain sugar; in the tetroses, C-3 is the key center. Compounds (II) and (IV), therefore, are classified as D-sugars, and (III) and (V) belong to the L-series. (II) and (III) are optical enantiomorphs but otherwise possess identical properties; they are, respectively, D- and L-erythrose. The ending *-ose* signifies a member of the sugar series and, in this instance the name erythrose is derived from the natural tetrahydric alcohol, erythritol, which is optically inactive by internal compensation; the "top half" and "bottom half" of the molecule are mirror images about an axis of symmetry which lies between C-2 and C-3. Erythritol is found free in lower plants and has been isolated from normal human urine (*3a*).



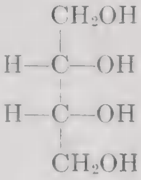
$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{H{-}C{-}OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

(VI)



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HO{-}C{-}H} \\ | \\ \mathrm{CH_2OH} \end{array}$$

(VII)



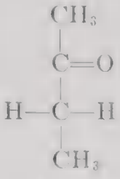
$$\begin{array}{c} \mathrm{CH_2OH} \\ | \\ \mathrm{H{-}C{-}OH} \\ | \\ \mathrm{H{-}C{-}OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

Erythritol

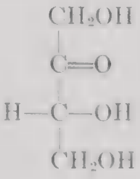
The two sugars (IV) and (V) are, respectively D- and L-threose and are termed epimers of the erythroses (Greek *epi* = above). Epimers differ from one another solely in respect of the "top" or lowest number asymmetric systems which, comparing (II) with (IV) and (III) with (V), are arranged in opposite stereochemical senses.

D-Erythrose (as its 4-phosphate) is the only member of this series so far known to be of natural origin (*4*).

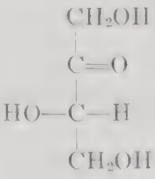
Related to the aldotetroses are two trihydroxy ketones (IX and X) which are derived from butan-2-one (VIII). These substances are un-



(VIII)



(IX)

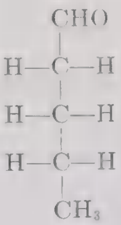


(X)

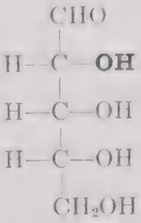
able to adopt any structure other than the one shown above and, strictly speaking, do not comply with the definition of a monosaccharide (see above). However, for a number of good reasons both chemical and biological, it is convenient to regard (IX) and (X) as simple sugars possessing a single asymmetric center, the nature of which is defined by its relation to D- or L-glyceraldehyde by the prefix D-glycero or L-glycero. Keto sugars, in general, are termed *-uloses*, the suffix being adopted from the archaic name for D-(—)-fructose, "levulose." Compound (IX) is thus D-glycerotetrulose or D-erythulose or D-threulose, the epimeric center, at C-2, differentiating erythroses and threoses having disappeared; (X) is, of course, the L-enantiomorph. D-Erythulose is known as its 4-phosphate and, in fact, is biologically interchangeable with D-erythrose 4-phosphate (5), with which it is isomeric. It may be noted that, in any class of monosaccharides all of which are based on the same number of carbon atoms, there are half as many ketoses as there are aldoses.

2. *Pentoses*

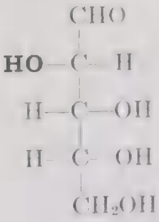
There are eight possible aldopentose structures, four D- and four L-; each sugar contains *three* asymmetric centers. Corresponding to the eight aldopentoses we find four ketopentoses (pentuloses), each of which contains *two* asymmetric centers. The aldoses are derived from pentanal (XI) and the pentuloses from pentan-2-one (XV). The D-series only is shown below. Epimeric pairs are (XII) and (XIII), (XVI) and (XVII), (XIV) and (XVIII). In the two pentuloses the asymmetric centers are best defined in relation to the aldotetroses which possess the same configuration; (XIV) is D-erythropentulose (cf. II) and



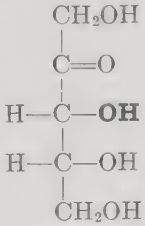
(XI)



(XII)
D-Ribose



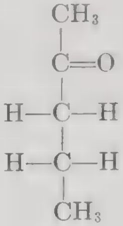
(XIII)
D-Arabinose



$$\begin{array}{c} \mathrm{CH_2OH} \\ | \\ \mathrm{C{=}O} \\ | \\ \mathrm{H{-}C{-}}\mathbf{OH} \\ | \\ \mathrm{H{-}C{-}OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

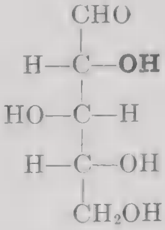
(XIV)

D-Erythropentulose
[D-Ribulose, -Arabinulose]



$$\begin{array}{c} \mathrm{CH_3} \\ | \\ \mathrm{C{=}O} \\ | \\ \mathrm{H{-}C{-}H} \\ | \\ \mathrm{H{-}C{-}H} \\ | \\ \mathrm{CH_3} \end{array}$$

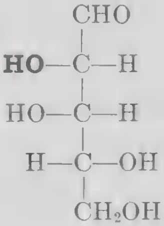
(XV)



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{H{-}C{-}}\mathbf{OH} \\ | \\ \mathrm{HO{-}C{-}H} \\ | \\ \mathrm{H{-}C{-}OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

(XVI)

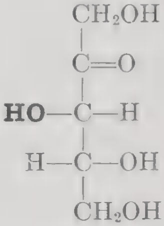
D-Xylose



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathbf{HO}\mathrm{{-}C{-}H} \\ | \\ \mathrm{HO{-}C{-}H} \\ | \\ \mathrm{H{-}C{-}OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

(XVII)

D-Lyxose

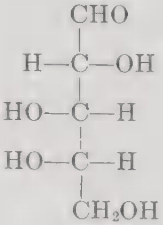


$$\begin{array}{c} \mathrm{CH_2OH} \\ | \\ \mathrm{C{=}O} \\ | \\ \mathbf{HO}\mathrm{{-}C{-}H} \\ | \\ \mathrm{H{-}C{-}OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

(XVIII)

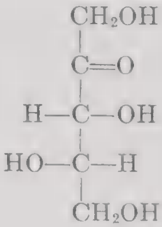
D-Threopentulose
[D-Xylulose, -Lyxulose]

(XVIII) is D-threopentulose (cf. IV). In common use, however, are such names as D-xylulose for (XVIII), which occurs, in small concentrations, as an intermediary metabolite (*6*). All these sugars can be shown experimentally to possess at C-4, the highest-numbered asymmetric center, the configuration of D-glyceraldehyde; all except D-lyxose (XVII) have been found in natural environments either as the free sugars or as compounds from which the free sugar may be obtained by hydrolysis. L-Enantiomorphs of arabinose (XIX) and of xylulose (L-threopentulose) (*7*) (XX) are the only members of the L-family of pentoses so far found in nature.



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{H{-}C{-}OH} \\ | \\ \mathrm{HO{-}C{-}H} \\ | \\ \mathrm{HO{-}C{-}H} \\ | \\ \mathrm{CH_2OH} \end{array}$$

(XIX)

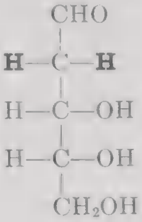


$$\begin{array}{c} \mathrm{CH_2OH} \\ | \\ \mathrm{C{=}O} \\ | \\ \mathrm{H{-}C{-}OH} \\ | \\ \mathrm{HO{-}C{-}H} \\ | \\ \mathrm{CH_2OH} \end{array}$$

(XX)

3. *2-Deoxy-*D*-ribose*

The monosaccharides so far described fulfill the historic condition that their elementary formula is $C_x(H_2O)_x$, which means that every carbon atom in the chain bears a covalently linked oxygen atom either in the form of hydroxyl or of carbonyl. Natural sugars are known which possess typical properties but have one or more carbon atoms in the chain bearing no hydroxyl but, in its place, a hydrogen atom. Such compounds are termed *deoxy* sugars and are given a numerical prefix to indicate which atom or atoms carry hydrogen in place of hydroxyl. Two natural deoxy pentoses have been found; the first is 2-deoxy-D-ribose (XXI) the sugar component of deoxyribonucleic acid (DNA) and of deoxyribonucleotides and -nucleosides.



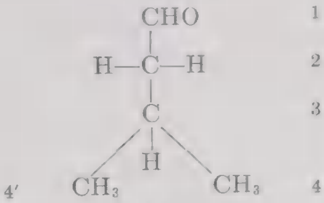
$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathbf{H}\text{—}\mathrm{C}\text{—}\mathbf{H} \\ | \\ \mathrm{H}\text{—}\mathrm{C}\text{—}\mathrm{OH} \\ | \\ \mathrm{H}\text{—}\mathrm{C}\text{—}\mathrm{OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

(XXI)

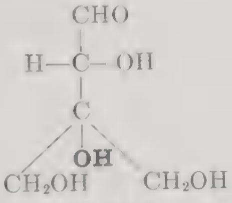
In general, the stereochemistry of deoxy sugars is defined by relating the asymmetric system to that of the aldose in which the same arrangement is found; (XXI) is therefore 2-deoxy-D-erythropentose. The second deoxypentose, cordycepose, is dealt with below.

4. *Branched-Chain Pentoses*

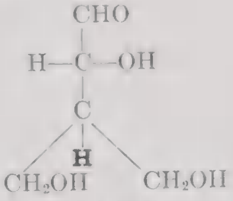
So far we have considered only sugars derived from unbranched hydrocarbon chains. Two natural five-carbon monosaccharides, derived from 3-methylbutanal (XXII), have been found in the form of glycosidic (q.v.) derivatives; from their chemical and physical behavior they must be regarded as pentoses. The sugars in question are D-apiose (XXIII) and cordycepose or 3-deoxy-D-apiose (XIV) (*8–10*).* The structures (XXIII) and (XXIV) possess but a single asymmetric center, at C-2. Complications of nomenclature, which are not encountered among the unbranched sugars, arise when certain derivatives of branched-chain sugars are considered; these complications will be discussed at a more appropriate time.



(XXII)



(XXIII)



(XXIV)

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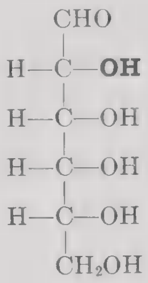
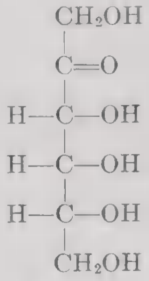
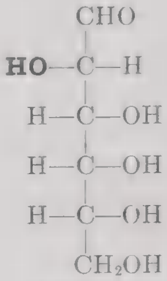
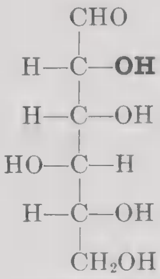
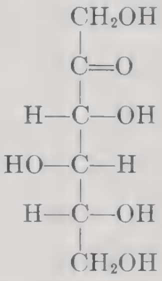
* Until recently D-apiose was known only in glycosides, found in members of the Umbelliferae [see Hudson (*8*)]. The (combined) sugar has also been found in the aquatic monocotyledon, *Posidonia australis* (Niadaceae) [see Bell *et al.* (*24*)]; in koksaghyz (Compositae) [see Chrastil (*8*)]; and in *Hevea brasiliensis* (Euphorbiaceae) [see Patrick (*8*)].

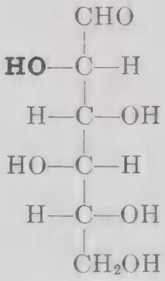

(XXV)
D-Allose

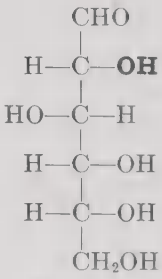

(XXVII)
D-Ribohexulose§
(D-Psicose)

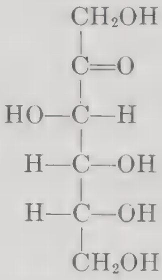

(XXVI)?*
D-Altrose‡

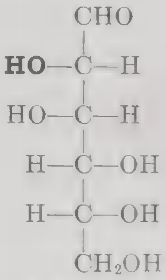

(XXVIII)
D-Gulose

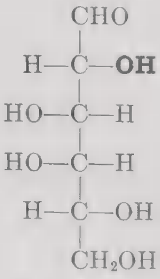

(XXX)
D-Xylohexulose
(D-Sorbose)

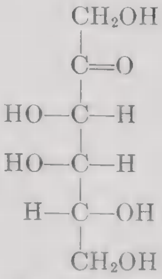

(XXIX)?*
D-Idose‡

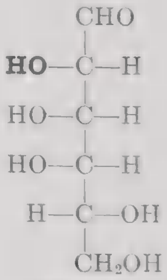

(XXXI)*
D-Glucose


(XXXIII)*
D-Arabinohexulose
(D-Fructose)


(XXXII)*
D-Mannose


(XXXIV)*
D-Galactose


(XXXVI)*
D-Lyxohexulose¶
(D-Tagatose)


(XXXV)*
D-Talose (*12*)

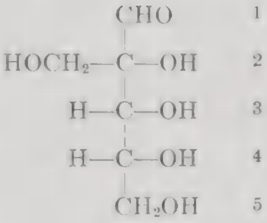
‡ The polysaccharide varianose, which is found in culture media of *Penicillium*

5. *Hexoses*

The pentoses can be considered as being derived from the tetroses by adding a H—C—OH grouping to their stack of asymmetric systems; addition of a similar system to the stack of asymmetric systems of the pentoses gives rise to the hexose series; each pentose yields two hexoses depending upon whether the added system is right- or left-handed. The D- and L-aldohexoses therefore consist of eight members each and the D- and L-hexuloses, four each. Of all these substances only a few have been so far found in nature. The D-family compounds are set forth, arranged in epimeric pairs alongside their related ketoses (XXV–XXVI); sugars that have been detected in natural environments are marked with an asterisk.

6. *Branched-Chain Hexoses*

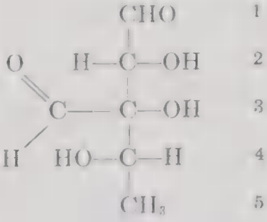
The earliest-known member of this series is D-hamamelose (2-*C*-hydroxymethyl-D-ribose) (*13*).



D-Hamamelose

The sugar is present in hamameli tannin (from *Hamamelis virginica*).

Streptose (*14*), a constituent of the hydrolysis products of the antibiotic streptomycin is 5-deoxy-3-*C*-formyl-L-lyxose, and is so far unique in possessing a side-chain carbonyl group:



Streptose

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varians is believed to be terminated by a radical derived from either D-idose or D-altrose.

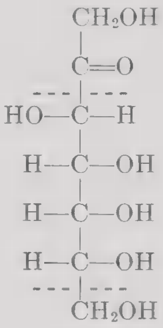
§ See reference 65.

¶ Obtained from hydrolyzate of gum from *Sterculia setigera* and from a lichen, *Rocella linearis* (*11*). Note that D-tagatose has not been detected as a component of gums from the following *Sterculia* spp.: S. *urens*, S. *tormentosa*, and *Brachychiton diversifolium* (formerly S. *caudata*).

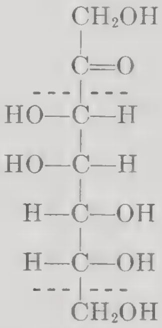
The writer is not certain whether to class this sugar with the pentoses or the hexoses.

7. *Heptoses and Octoses*

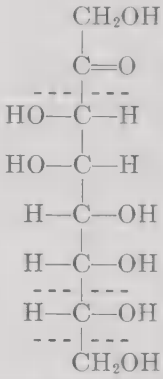
A few heptoses and at least one octose have been found as free sugars in plant extracts. Of the seven carbon sugars, two heptuloses D-altroheptulose (sedoheptulose) (XXXVII) (*15*) and D-mannoheptulose (mannoketoheptose) (XXXVIII) (*16*) have been known for a considerable time. The first occurs in especially large amounts in plants of the *Sedum* family, but its phosphate has recently been detected as an intermediary in carbohydrate metabolism of a wide variety of organisms. D-Mannoheptulose is found in the avocado pear, in which its phosphate also occurs.



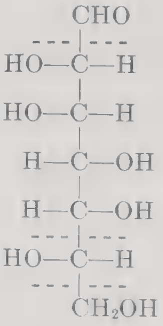
(XXXVII)



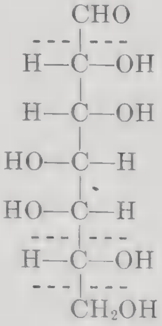
(XXXVIII)



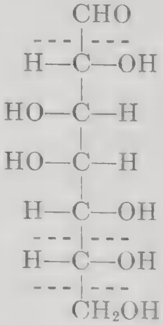
(XXXIX)



(XLII)



(XLI)



(XL)

The systematic names are based on the configuration of the stack of asymmetric centers at carbons 3, 4, 5, 6, which are the same, respectively, as those of D-altrose (XXVI) and D-mannose (XXXII).

D-Glycero-D-galactoheptose (XL), an aldoheptose, has been found among the hydrolysis products of a specific bacterial polysaccharide (*Chromobacterium violaceum*) (*17*); D-glycero-L-mannoheptose (XLI) very probably occurs in cultures of *Shigella flexneri* (*18*), and its optical enantiomorph L-glycero-D-mannoheptose (XLII) has been identified in the lipoglycoprotein complex of the cell wall of *Escherichia coli* B (*19*).

The first natural octose, D-glycero-D-mannoctulose (XXXIX) has recently been isolated from an aqueous extract of the Californian avocado (*20*) [the well-known source of D-mannoheptulose (XXXVIII)] and, as will be seen from its formula, is related in structure. It also occurs in *Sedum* spp. (*20a*).

8. *Monosaccharides as Heterocylic Compounds*

Although we have up to this point considered the monosaccharides as open-chain compounds, it must be emphasized that they rarely, if ever, exist free and unsubstituted in this condition. As solids and in solutions, except at extremes of pH, they behave chemically and physically as substances devoid of carbonyl groups in any appreciable amount. Neither infrared nor ultraviolet spectroscopies indicate the presence of the $>C{=}O$ system. Moreover, they will react reversibly, under suitable conditions, with equimolar proportions of alcohols, phenols, and suitable nitrogen bases, through the elimination of equimolar amounts of water, to form glycosyl derivatives. (The glycosyl radical is $[C_x(H_2O)_x]$ minus OH; cf. CH_3OH and CH_3-). These glycosyl derivatives are no longer capable of reducing, as do free sugars, alkaline solutions of polyvalent cations or anions such as Cu^{2+}, Bi^{3+}, Ce^{4+}, or $Fe(CN)_6^{3-}$.* Moreover, the glycosides do not react with carbonyl reagents nor do they undergo oxidation ($>C{=}O \rightarrow -CO_2H$) nor reduction ($>C{=}O \rightarrow >CHOH$). Hence the aldehyde group of the aldoses, and the ketone grouping of the ketoses, must have become transformed in the generation of the glycosyl derivatives.

When a crystalline reducing sugar (aldose or ketose) is dissolved in an ionizing solvent, the optical activity of the solution changes progressively from an initial level to a steady value; this phenomenon is termed mutarotation. In a number of instances, two physically distinct crystalline modifications of a single sugar can be obtained that differ in their initial optical rotatory power on being dissolved; on mutarotating, both modifications ultimately attain the same equilibrium value. For example, the so-called "α" and "β" D-glucoses, when dissolved in water show, respectively, initial $[\alpha]_D$ values of $+110°$ and $+19°$ and both ultimately attain the equilibrium value of $+52.5°$.

All the aforementioned phenomena are consistent with the possession by the sugar of a ring structure in which an intramolecular rearrangement has altered the carbonyl system so that an internal cyclic hemiacetal (or hemiketal) is formed between the carbonyl group and a hydroxyl group attached to a carbon atom of higher number. In such a structure a new asymmetric center is generated out of the original carbonyl-carbon atom of the open-chain structure, and, as a result, two distinct cyclihemiacetals (the "α" and "β" modifications) can be formed by this cyclization. Cyclizations of aldehydo-D-glucose (XLIII) and keto-D-fructose (XLIV) are illustrated here.

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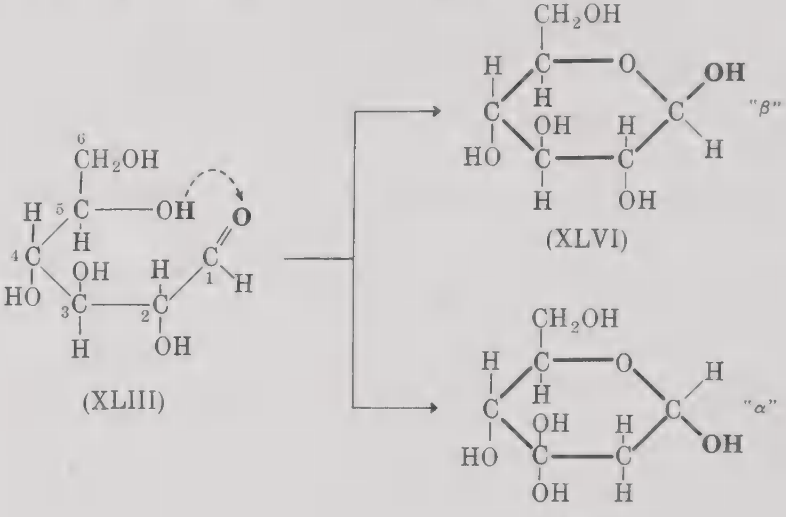
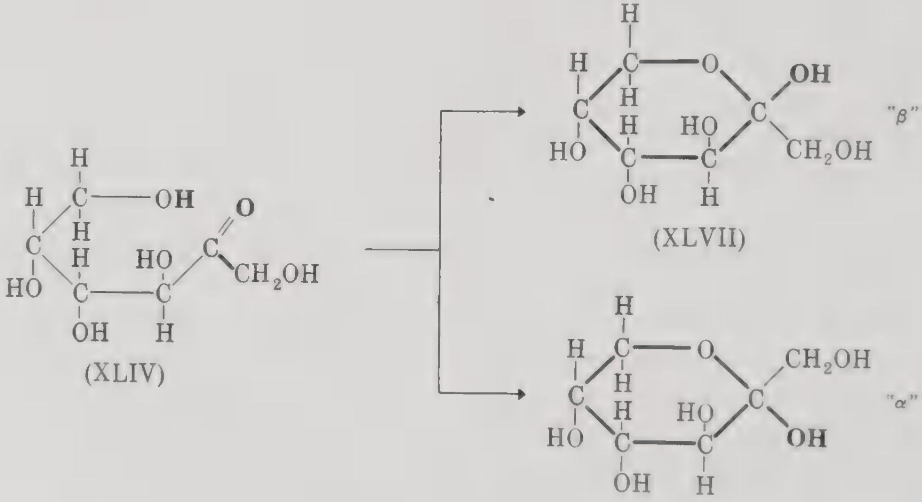
* Ketoses reduce these reagents because they have the structure in the open-chain form of α-hydroxy ketones.

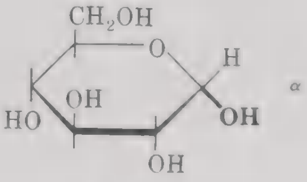


(XLIII) (XLVI) (XLV)

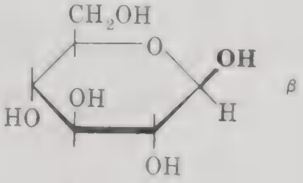


(XLIV) (XLVII) (XLVIII)

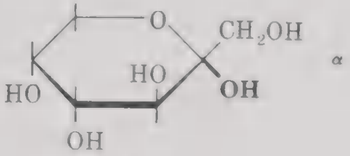
The cyclic formulas (XLV–XLVIII) may be represented in the simplified manner devised by Haworth (XLVa–XLVIIIa).



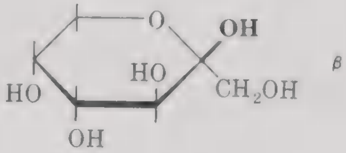
(XLVa)



(XLVIa)



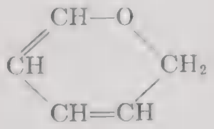
(XLVIIIa)



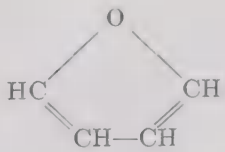
(XLVIIa)

The six-membered rings are represented as flat; although this is not strictly true (*21*, *21a*), it is convenient for the present purposes of structural comparison to use Haworth's formulation and to talk of hydroxyl groups being "above" and "below" the "plane" of the ring. The structures (XLVa and XLVIa), for α-D-glucose and β-D-glucose, and (XLVIIIa and XLVIIa) for α- and β-D-fructose, are sufficient to indicate the approximate relative positions of the hemiacetal hydroxyls (heavy type) in the α- and β-modifications of the sugars as they have been defined experimentally. The α- and β-forms of a sugar are, of course, not optical isomers; they differ only with respect to the stereoisomerism of the asymmetric system generated by cyclic hemiacetal formation about the carbon atom of the reducing group (C-1 in aldoses and C-2 in ketoses). This phenomenon has been named *anomerism* and the α- and β-isomers are termed *anomers* (*21b*).

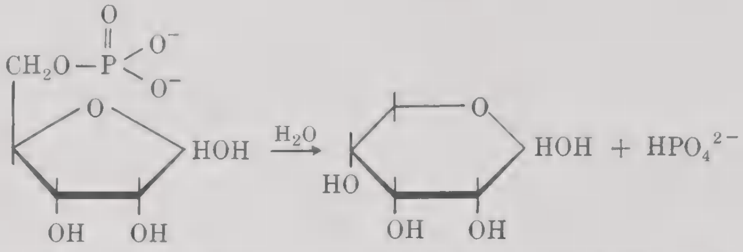
The six-membered sugar structures are termed *pyranoses*, after pyran:



An alternative ring structure is possible, formed from open-chain monosaccharides, to give a five-membered cyclic hemiacetal. Here the ring, which can only be flat, is termed *furanose* after furan.



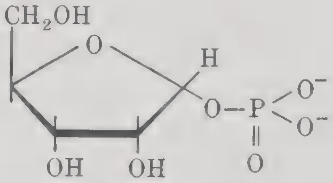
Many derivatives of monosaccharides are known where the substituting group either replaces the reducing group —OH or else occupies a hydroxyl group in such a position that the ring cannot expand to adopt the more stable pyranose form (Reaction 1)



α/β-D-Ribofuranosyl-
5-phosphate

α/β-D-Ribopyranose + phosphate^{2-}

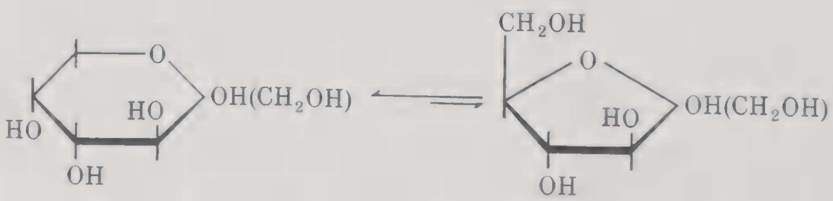
(1)



α-D-Ribofuranosyl phosphate

Ribofuranose rings can exist in a stable condition only when the sugar is substituted in such a way as to prevent enlargement of the ring, e.g., by substitution at the hydroxyl on C-5 or on C-1.

Aqueous solutions of certain monosaccharides, especially D-fructose contain an appreciable amount of the furanose form (glucose is a notable exception). The amount of furanose sugar present is usually dependent upon the temperature and effects the equilibrium [α] (*22*) (Reaction 2).

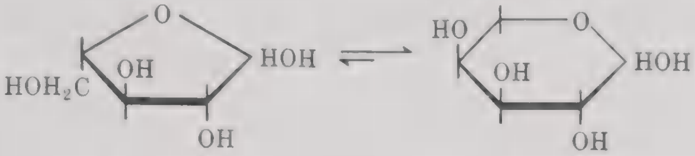


α/β-D-Fructopyranose

α/β-D-Fructofuranose

(2)

It is noteworthy that in every known natural derivative of D-fructose the sugar moiety has the furanose structure; this is also the case in the vast majority of derivatives of L-arabinose, which, in solution, also exists to a considerable extent as the five-membered ring (Reaction 3).



(3)

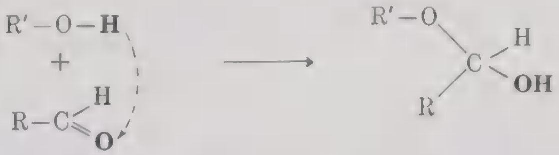
α/β-L-Arabofuranose α/β-L-Arabopyranose

It may be pointed out that in both fructose and arabinose (it is immaterial whether D- or L-configurations are considered) both the furanose rings and the pyranose rings have the same relative arrangements of hydroxyl groups.

B. GLYCOSIDES

As has already been mentioned, the hydrogen of the hydroxyl group associated with the "reducing group" of the cyclic forms of monosaccharides (C-1 in aldoses and C-2 in ketoses) can undergo substitution by a number of radicals. These radicals can be alkyl, aryl, acyl, arylacyl, phosphate, etc. In theory, and frequently in practice, two anomeric forms (α- and β-) of each derivative can exist which are structurally related to the α- and β-anomers of the parent sugar. Compounds derived from sugars by this kind of substitution, and by this kind alone, are termed *glycosides*, and it is through glycoside formation by appropriate enzymatic action that a vast number of sugar derivatives have originated in living organisms.

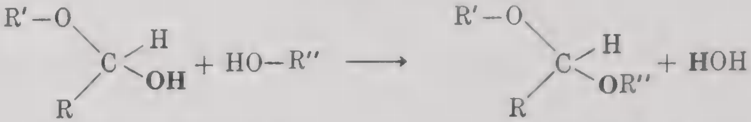
The glycosides may be compared, in respect of their chemical properties, with the acetals that are formed by water elimination between an aldehyde and two molecules of an alcohol in a two-stage process, the first of which resembles the formation of the sugar ring from the open chain (Reaction 4).



(4)

Alcohol + Aldehyde Hemiacetal

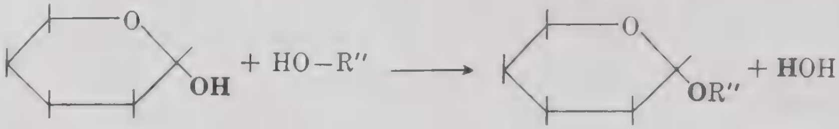
This reaction is an intermolecular redistribution of covalencies by transfer of a hydrogen atom without elimination of water. The second phase in acetal formation does involve water elimination and is a substitution of the hydrogen marked in heavy type (Reaction 5).



(5)

Hemiacetal + Alcohol Acetal + Water

Glycoside formation may be expressed in the same way (Reaction 6).

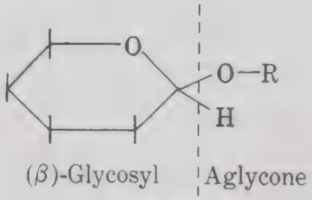


(6)

α-Aldopyranose + alcohol α-Aldopyranoside + water

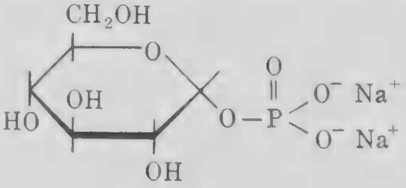
Few, if any natural glycosides are known where R″ is a simple alkyl radical;* however, very many alkyl glycosides have been prepared synthetically both by inorganic and enzymatic catalysis. On the other hand, a vast number of glycosides from plant (mainly) and animal sources have been discovered which are derived from phenolic molecules and alicyclic alcohols.

Glycosides are made up of two parts: (*a*) the parent sugar part, and (*b*) that part originating in the molecule with which the sugar was condensed. These parts are termed, respectively, the *glycosyl* radical (α- or β-) and the *aglycone* (XLIX).

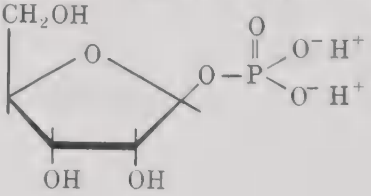


(XLIX)
(β)-Aldopyranoside

In the so-called "aldose-1-phosphates" the aglycone is derived from orthophosphate; the glycosides can exist in both α- and β-anomeric forms, and it is most logical to name them as glycosides (L, LI).



(L)
α-D-Glucopyranosyl
disodium phosphate



(LI)
β-D-Ribofuranosyl
dihydrogen phosphate

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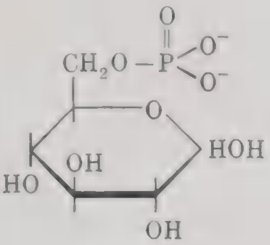
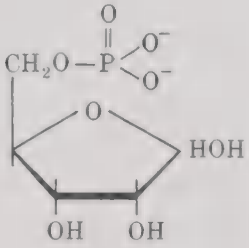
* The ethyl α-D-galactoside isolated from sweet yellow lupin [by Nottbohm and Mayer (23)] may have arisen through transglycosylation with the ethanol used in the extraction.

Naming the "1-phosphates" as glycosides distinguishes them clearly from the isomeric *esters* (LII, LIII).



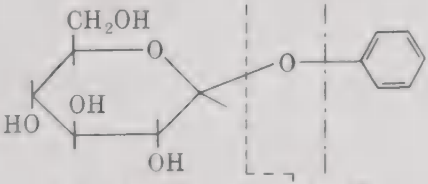
(LII)
α/β-D-Glucopyranose-
6-phosphate



(LIII)
α/β-D-Ribofuranosyl-
5-phosphate

In the two esters the *-ose* nomenclature emphasizes the fact that both substances behave as reducing sugars and that the phosphate moiety of the molecule is not attached to the reducing group of the sugar. Moreover, it serves as a reminder that the chemical behavior of the glycosidic phosphates differs from that of the phosphoric esters.

It is perhaps worth pointing out that glycosides, such as those formed between a sugar and a phenol can be named in several ways. Taking the example of the β-glucoside derived from β-glucose and phenol, which is usually termed phenyl-β-D-glucopyranoside, we can also have (LIV).

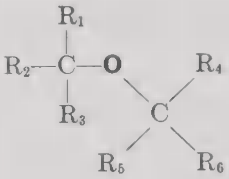


O-β-D-Glucopyranosyl phenol
β-D-Glucopyranosyloxy phenyl

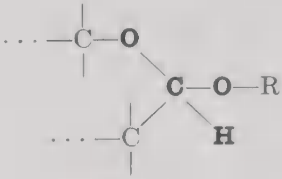
(LIV)

The difference between the glycopyranosyl and glycopyranosyloxy radicals should be noted.

To conclude this section it must be emphasized that the glycosidic system is not an ether; glycosides are readily hydrolyzed by dilute acid (pH 0 for pyranosides and pH 2–3 for furanosides) and, in the instances of some glycosides in which the aglycone is phenolic, by dilute alkali. Ordinary ethers are not appreciably attacked; their structures (LV, LVI) are compared.



(LV)
Ether



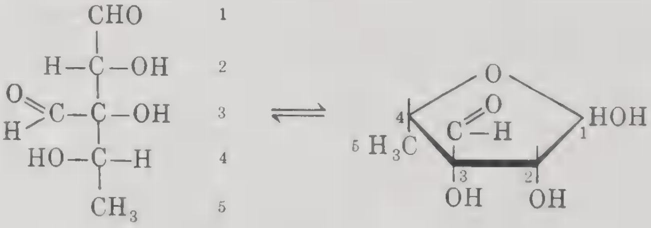
(LVI)
Glycoside

In the ether, R_1–R_6 are either hydrogen or carbon whereas one of the carbons (heavy type) in the glycoside is attached to *two* oxygen atoms, and is, as has already been pointed out, the functional carbon of an acetal system.

C. Ring Formation in Branched-Chain Monosaccharides

Some special attention must be paid to the behavior of the branched-chain sugars when they form rings, as indeed they must do in their natural environments, since they are found only in glycosidic combination, or as esters in the case of hamamelose.

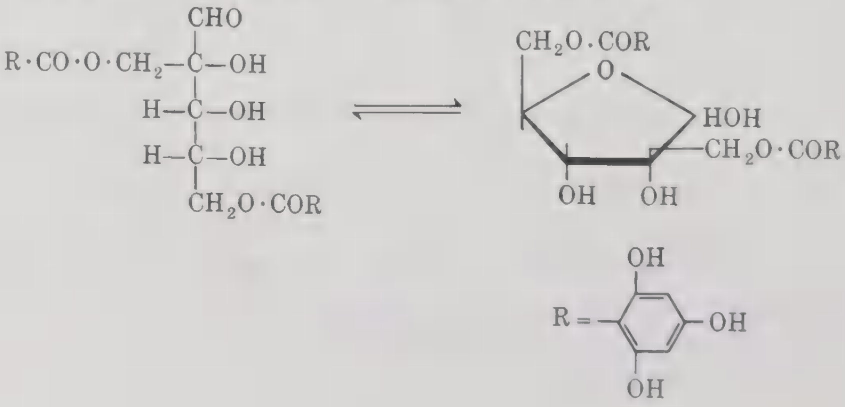
Streptose (Section I, A, 6) can exist as a ring only in the furanose form since there is no hydroxyl group available with which to form a pyranose ring (Reaction 7).



(7)

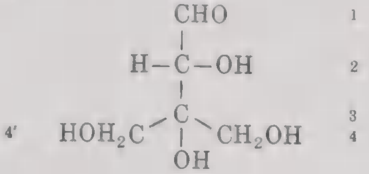
Aldehydo-D-streptose α/β-D-Streptofuranose

Hamamelose (Section I, A, 6), which occurs as the 2,5-digalloyl ester, could be either in a furanose ring or open chain. The free sugar can form either pyranose or furanose rings (Reaction 8).

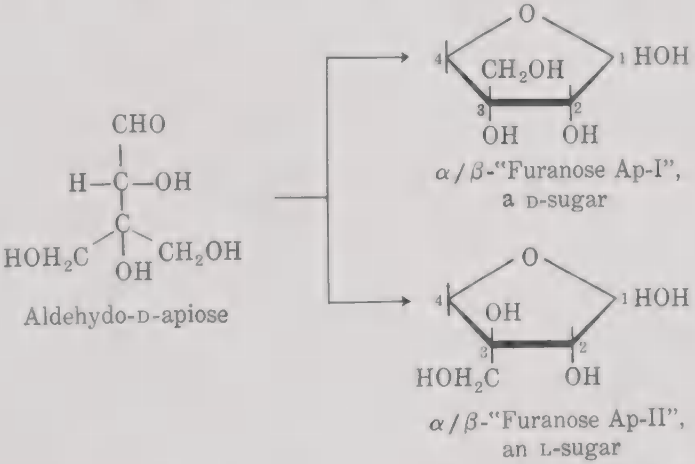


(8)

The example of apiose (*8*), which is found only in glycosidic union, presents considerable difficulties. Formulated in the aldehydo form the natural isomer belongs to the D-series.



C-2 is the highest numbered (and only) asymmetric center; it has been proved to have the configuration of D-glyceraldehyde. Apiose can form only a furanose ring, as it must when in glycosidic combination. When it forms such *a ring a new asymmetric center is generated at C-3*. This new asymmetric center must, by definition, define the stereochemical family to which the sugar, in its ring form, is to be assigned. But, since the new center may have either a D- or an L-configuration, and as at present we have no means of telling which is, in fact, the correct configuration in a glycosidic derivative of apiose, we cannot properly describe such a compound (Reaction 9).


Aldehydo-D-apiose

α/β-"Furanose Ap-I", a D-sugar

α/β-"Furanose Ap-II", an L-sugar

(9)

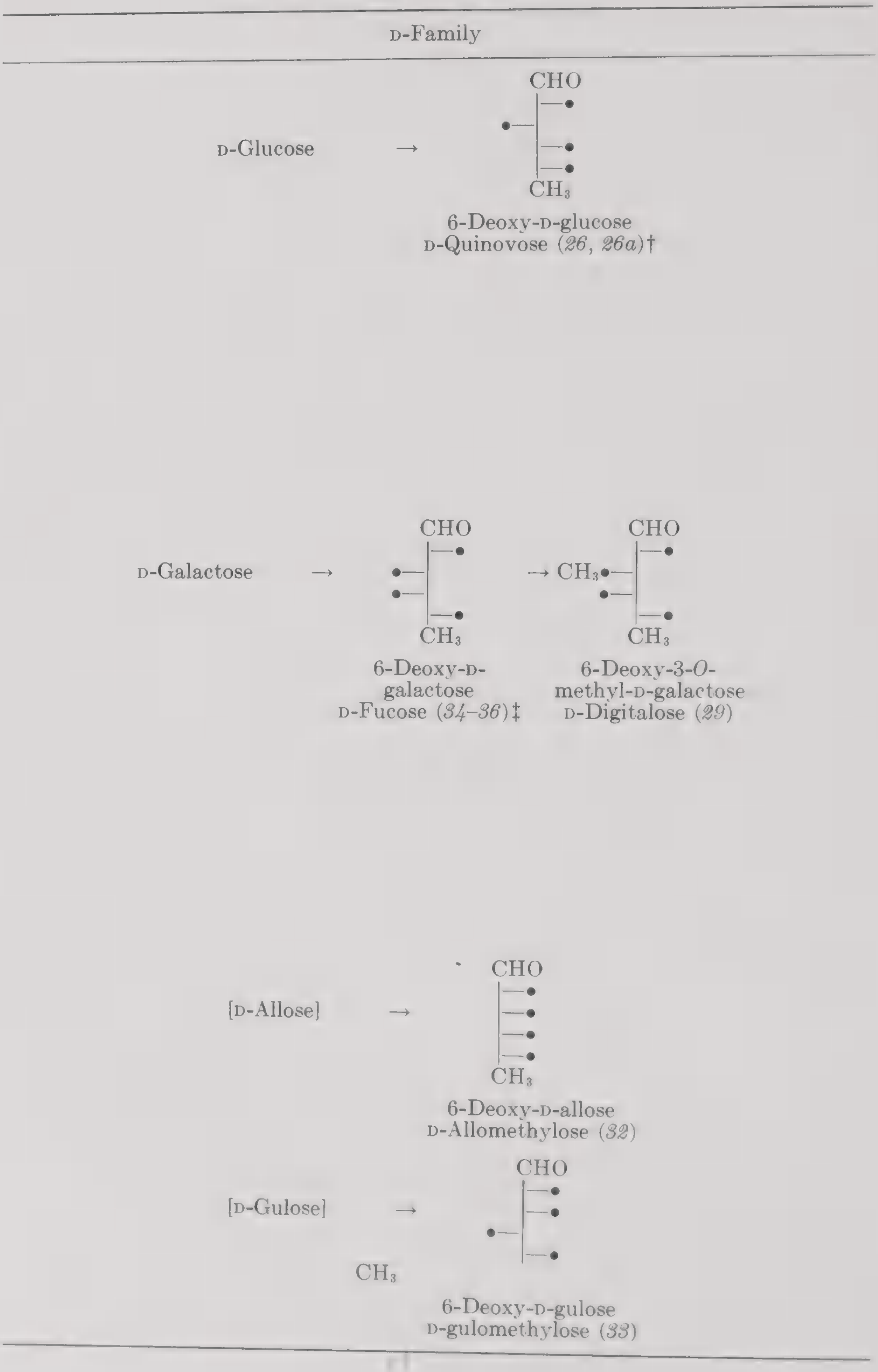
"Ap-I" and "Ap-II" are not mirror images, but are related in a manner analogous to, but not identical with, α-β anomerism. "Ap-I" could be named 3-*C*-hydroxymethyl-D-erythrofuranose and "Ap II", 3-*C*-hydroxymethyl-L-threofuranose. But such names can in no way be related to the name D-apiose. The question has been discussed and some tentative suggestions have been put forward (*24*).

A similar situation exists in the case of cordycepose (*9*, *10*).

D. THE DEOXYHEXOSES (*25*)

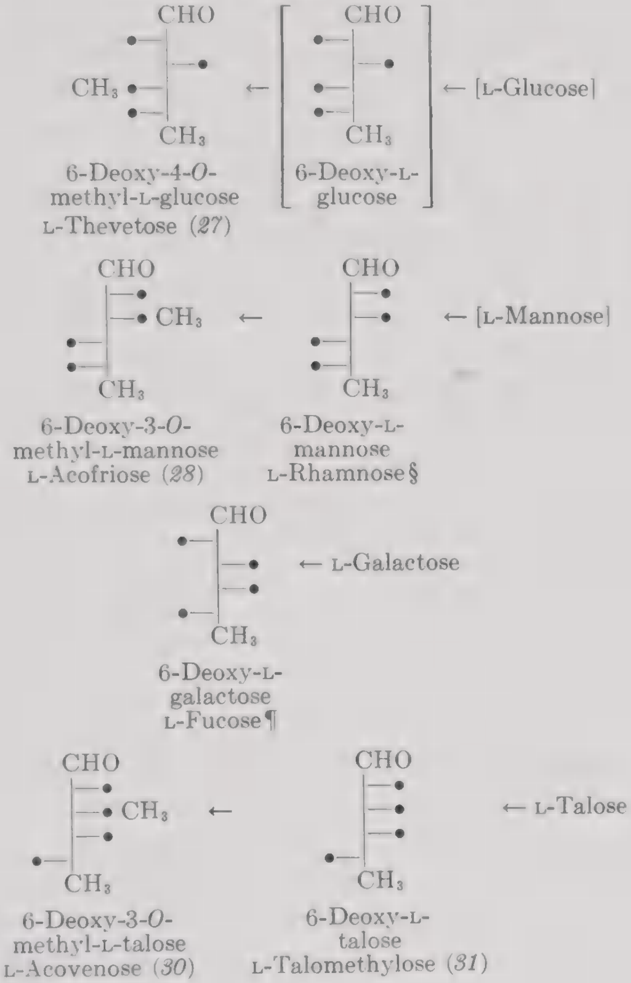
A number of natural hexoses are known where the primary alcoholic group of C-6 is reduced to —CH_3. In addition some dideoxy hexoses

SCHEME I


D-Family
D-Glucose
CHO
CH$_3$
6-Deoxy-D-glucose
D-Quinovose (*26, 26a*)†
D-Galactose
CHO
CH$_3$
6-Deoxy-D-galactose
D-Fucose (*34–36*)‡
CHO
CH$_3$
CH$_3$
6-Deoxy-3-*O*-methyl-D-galactose
D-Digitalose (*29*)
[D-Allose]
CHO
CH$_3$
6-Deoxy-D-allose
D-Allomethylose (*32*)
[D-Gulose]
CHO
CH$_3$
6-Deoxy-D-gulose
D-gulomethylose (*33*)

SCHEME (I) *Continued*

L-FAMILY



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† The sugar has been variously called D-isorhamnose, D-epirhamnose, isorhodeose, and chinovose. It is found in glycosides from *Cincona* spp. (Rubiaceae) (*26*). D-Quinovose has, until recently, been associated with the plant kingdom. Hydrolyzates of the venom of the holothurian (sea cucumber) *Actinopyga agassizi* yield the sugar along with D-xylose, 3-*O*-methyl-D-glucose and D-glucose itself (*26a*).

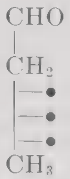
‡ The sugar is found in glycosidic combination, along with D-glucose and L-rhamnose in glycosides obtained from members of the Convolvulaceae (*34*).

§ See Section V,K.

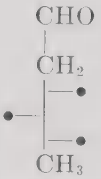
¶ See Section V,H.

occur in nature; in these a secondary alcoholic group is reduced to —CH$_2$— while C-6 is also —CH$_3$. No deoxy sugar has been found in an unsubstituted state except in traces; all occur in glycosidic linkage in one type of compound or another. With the exceptions of L-fucose (6-deoxy-L-galactose) and L-rhamnose,* which occur in many other situations, the monodeoxyhexoses are found only in the so-called cardiac glycosides and their chemical relatives in which the aglycones are polycyclic. Some of the deoxyhexoses occur as monomethyl ethers where a secondary hydroxyl has become methylated. The structural relationships of the 6-deoxyhexoses are illustrated in Scheme I; the diagram must on no account be taken to indicate any biological routes by which these sugars might be produced. The simplified formulas of Reichstein are used and are self-explanatory. Compounds within square brackets have not been found in a biological environment.

Two 2,6-dideoxyhexoses occur in certain glycosides. They are D-digitoxose and D-boivinose which are formulated, the stereochemistry of the three asymmetric centers being described by a prefix indicating their relationship to the pentose series.

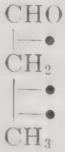


D-Digitoxose (*37*)
(2,6-Dideoxy-D-ribohexose)

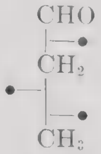


D-Boivinose (*38*)
(2,6-Dideoxy-D-xylohexose)

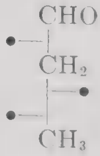
Four 3,6-dideoxyaldohexoses have been isolated from lipopolysaccharides of various bacteria and have been named, abequose (*38a*), tyvelose (*38b*), colitose (*38c*), and paratose (*38d*). A fifth member of this series, ascarylose (*38e*) is present as glycosidic lipids in the eggs of the ascarid *Paraascaris equorum*.



Paratose
(D-*erythro*-D-Glycero-
3,6-dideoxyhexose)

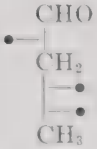


Abequose
(D-*threo*-D-Glycero-
3,6-dideoxyhexose)

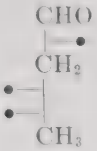


Colitose
(L-*threo*-L-Glycero-
3,6-dideoxyhexose)

* L-Fucose is converted into L-fuculose (6-deoxy-L-tagatose) by an enzyme from a mutant strain of *Escherichia coli* [Green and Cohen (*25a*)]; L-rhamnose is likewise isomerized into L-rhamnulose (6-deoxy-L-fructose) by *Pasteurella pestis* [Englesberg (*25a*)]; and by *E. coli* [Wilson and Ajl (*25a*)].



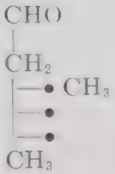
Tyvelose
(D-*threo*-L-Glycero-
3,6-dideoxyhexose)



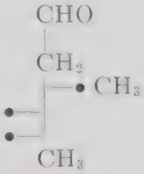
Ascarylose
(L-*erythro*-D-Glycero-
3,6-dideoxyhexose)

Abequose with colitose, and tyvelose with ascarylose, form enantiomorphic pairs (*38f*).

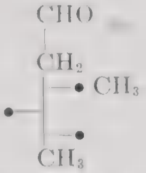
Allied to digitoxose and boivinose are the four known 2,6-dideoxy-3-*O*-methylhexoses which occur in steroid glycosides D-cymarose, L-oleandrose, D-sarmentose, and D-diginose (cf. Section V, N).



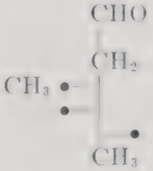
D-Cymarose (*39*)
2,6-Dideoxy-
3-*O*-methyl-D-
ribohexose



L-Oleandrose (*42*)
2,6-Dideoxy-
3-*O*-methyl-L-
arabinohexose



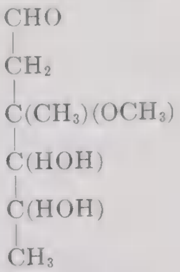
D-Sarmentose (*40*)
2,6-Dideoxy-
3-*O*-methyl-D-
xylohexose



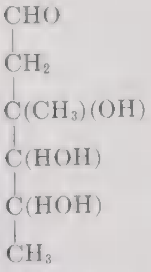
D-Diginose (*41*)
2,6-Dideoxy-
3-*O*-methyl-D-
lyxohexose

E. Branched Deoxyheptoses

Among the sugars that have been isolated from the hydrolytic fragmentation of certain antibiotics (*42a*) of fungal origin are three branched-chain deoxyheptoses. At the date of writing, the stereochemistry of the first two, cladinose (*43*) and mycarose (*44*) (from erythromycin and magnamycin, respectively) have not been established; their generalized formulas are shown.

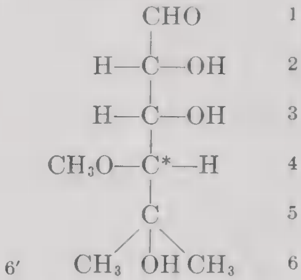


Cladinose
2,6-Dideoxy-3-*C*-methyl-
3-*O*-methylhexose



Mycarose
2,6-Dideoxy-3-*C*-methyl-
hexose

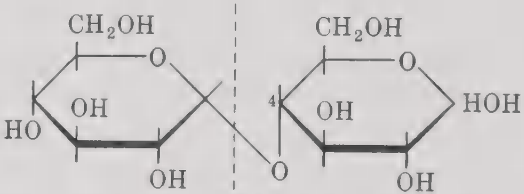
The third branched-chain sugar is the dideoxyheptose, noviose, which is the aldose moiety of novobiocin and has been given the structure shown, based on L-lyxose (*45*). The highest-numbered asymmetric center is C-4 (marked by an asterisk).



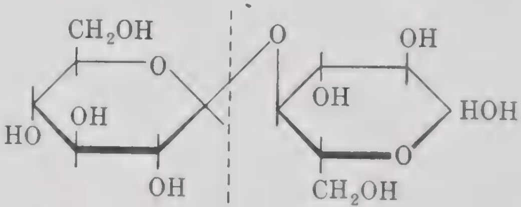
Noviose
5,5-Di-*C*-methyl-4-*O*-methyl-L-lyxose

II. Intersugar Glycosidic Compounds: Disaccharides and Oligosaccharides

Although a vast company of aryl, and other glycosides is known at the present time, the biochemist usually has greater interest in those glycosides in which a monosaccharide takes the place of the customary noncarbohydrate aglycone. For example, the sugars maltose and cellobiose, being formed from two monosaccharide parts, are known as disaccharides and are exactly comparable with the pair methyl-α- and methyl-β-D-glucopyranosides:



4-*O*-α-D-Glucopyranosyl α / β-D-Glucopyranose
α / β-Maltose



4-*O*-β-D-Glucopyranosyl α / β-D-Glucopyranose
α / β-Cellobiose

In order to save space in describing di- and higher saccharides or "oligosaccharides," a shorthand nomenclature is convenient. The term oligosaccharide (Greek *oligos* = few) is usually applied to complex sugars containing a known number of monosaccharide units. Since there are various modifications of such shorthand descriptions, it is usually necessary to provide a clue for the reader. The following examples will suffice to explain the system used in this chapter.

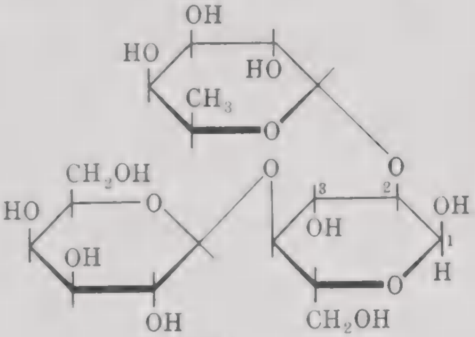
a. Shorthand descriptions for β-maltose and α-cellobiose: β-Maltose is 4-*O*-α-D-glucopyranosyl-β-D-glucopyranose, or

Gα4Gβ

where G = D-glucopyranose and α, β, and the numbers have their usual significance. α-Cellobiose is 4-*O*-β-D-glucopyranosyl-α-D-glucopyranose, or

Gβ4Gα

b. The trisaccharide shown here (LVII) is described as 2-*O*-α-L-fucopyranosyl-(4-*O*-β-D-galactopyranosyl)-α-D-glucopyranose. In shorthand symbols the description becomes



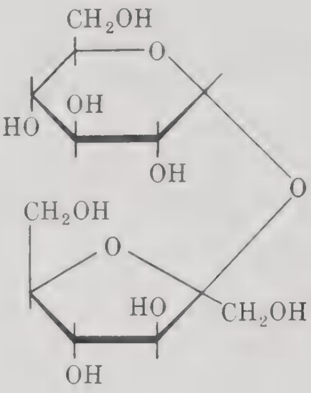
(LVII)

L - Fu
α
2
Gaβ4Gα

where L-Fu is L-fucopyranose; Ga is D-galactopyranose; and G is D-glucopyranose. The D prefix is omitted for simplicity.

The well-known nonreducing disaccharides sucrose and trehalose are composed of two monosaccharide units joined in mutual glycosidic linkage which involves both their reducing groups.

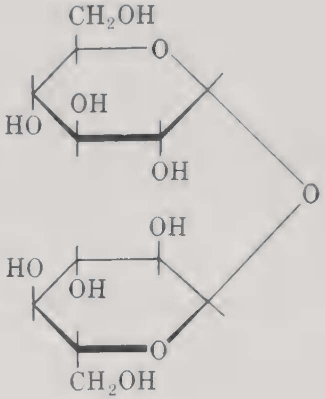
It is this capacity to unite monosaccharides one with another that enables the living cell to elaborate oligo- and polysaccharides; the structures of many of these substances have been defined through chemical and enzymatic study. The more complex saccharides are extremely difficult to describe in any concise manner; in this field shorthand symbols will be found of great utility.



Sucrose

α-D-Glucopyranosyl-β-D-fructofuranoside
or
β-D-Fructofuranosyl-α-D-glucopyranoside
Symbol: GαβFf

(Ff = D-fructofuranose)



Trehalose

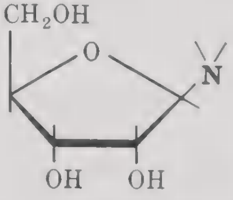
α-D-Glucopyranosyl-α-D-glucopyranoside

Symbol: GααG

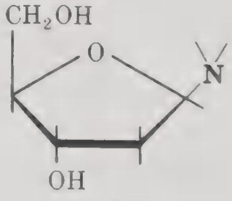
III. Nucleosides, Nucleotides, and Nucleic Acids (46)

A. DERIVATIVES OF D-RIBOSE AND 2-DEOXY-D-RIBOSE

The aglycones of glycosides which have been considered above have been derived from hydroxy compounds. A universally distributed group of glycosides is composed of compounds in which the pentoses D-ribofuranose (LVIII) and 2-deoxy-D-ribofuranose (LVIX) are linked through their reducing groups to a nitrogen atom in the β-anomeric configuration.

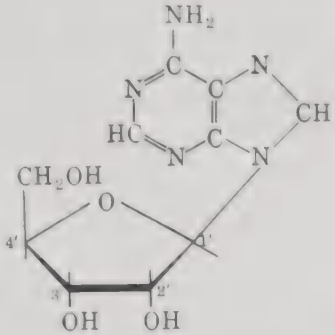


(LVIII)



(LIX)

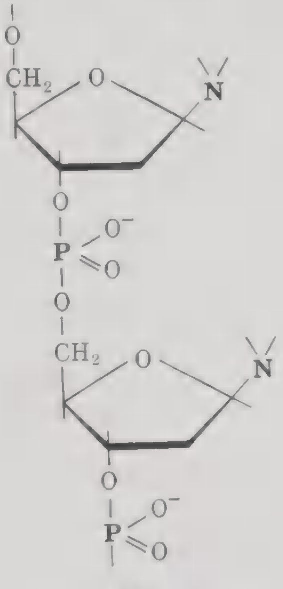
The nitrogen joined to the glycosyl radical in these compounds is usually that occupying position 9 in purines or position 3 in pyrimidines. Such compounds are classed as *nucleosides*, more specifically as ribonucleosides and deoxyribonucleosides. When an actual compound is considered, e.g., the derivative formed between adenine and D-ribose, the purine (or pyrimidine) base is numbered as usual and the numbers



(LX)

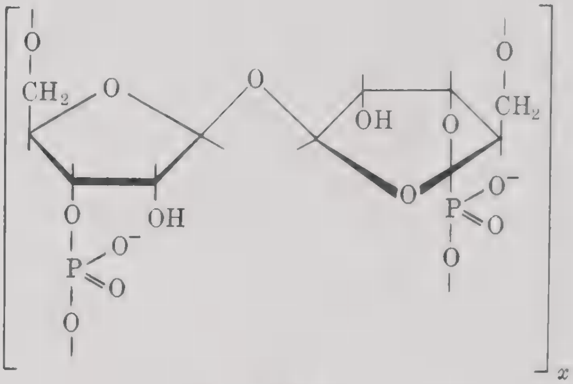
of the carbon atoms in the sugar are given primes, i.e., 1′, 2′, etc. The nucleoside illustrated (LX) is 9-adenyl-β-D-ribofuranoside. Nucleosides of both D-ribose and 2-deoxy-D-ribose frequently occur esterified at C-5′ or C-3′ by phosphate. Such compounds are termed *nucleotides*, or more specifically, ribotides or deoxyribotides.

The nucleic acids are constituted from large numbers of ribo- or deoxyribonucleotides linked in chains through phosphate (LXI).



(LXI)

It is not intended, in this chapter, to consider these substances to any great extent, as they are fully dealt with elsewhere. The foregoing structures have been given in order to offer a comparison between the "*O*-glycosides" and their "*N*" analogs. It may be noted that neither D-ribose nor 2-deoxy-D-ribose have ever been detected in any form of combination other than *N*-glycosidic with the single exception of a poly(diribofuranose phosphate) (*47*) isolated from *Hemophilus influenzae* type B and believed to have a structure such as that shown (LXII).



(LXII)

B. Nucleosides Derived from Sugars Other Than d-Ribose or 2-Deoxy-d-ribose

The Caribbean sponge (*Cryptothetia crypta*) has been shown to contain two nucleosides based on the rare sugar D-arabinose (XIII) in the furanose form (*48*). This sugar has hitherto been found only in glycosidic combination in certain bacteria (*48a*)* and among the products of prolonged *oxidative* hydrolysis of compounds (? glycosides) obtained from plants of the genus *Aloe* (*48b*). The arabinonucleosides are derived from thymine and uracil and have been named, respectively, spongothymidine and spongouridine; in the sponge they are accompanied by a D-riboside of 2-*O*-methyl-6-amino purine.

From the culture medium of the interesting mold *Cordyceps militaris* a nucleoside of D-cordycepose (*9, 10*) and adenine has been isolated. The configuration (D- or L-) of the cordycepofuranose moiety of this substance is at present unknown (cf. Section I,C). The ketohexose, D-psicose, hitherto not positively identified as a natural sugar, has recently been proved to be synthesized by *Streptomyces hygroscopicus* var. *decoyinine*, which produces an antibiotic substance consisting simply of psicofuranosyl adenine (*49*). The substance is represented in the β-anomeric form (LXIII); this has not so far been established.

The structure of the D-psicofuranosyl radical is closely similar to that of D-ribofuranosyl, in that a hydroxymethyl group replaces the hydrogen atom attached to C-1. D-Psicose is, of course, D-ribohexulose (D-allulose or -altrulose, XXVII).

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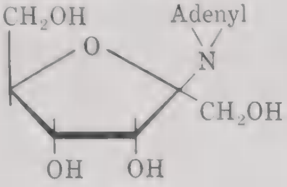
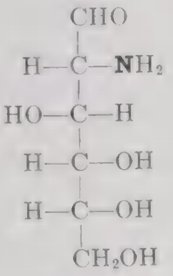
* In polysaccharides of *Mycobacterium tuberculosis* and of *Nocardia asteroides* (*48a*).



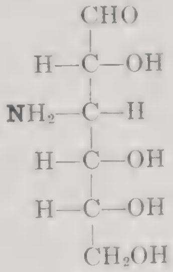
(LXIII)

IV. Aminodeoxy Monosaccharides, "Amino Sugars" (50)

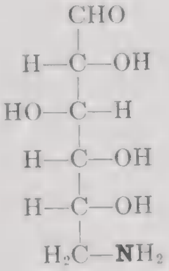
Two sugars, widely distributed especially in the animal kingdom, are structurally derived from D-glucose and D-galactose by replacement of the hydroxyl of C-2 by the amino group. The sugars, which have not been found except in glycosidic combination, are D-glucosamine (2-amino-2-deoxy-D-glucose) (LXIV) and D-galactosamine (2-amino-2-deoxy-D-galactose) (LXVIII). For long no natural amino sugars other than these two were known, but improved methods of analysis, and the interest aroused in microorganisms and their metabolic products, has led to the discovery of a variety of hitherto unsuspected amino sugars. From the antibiotic kanamycin, on hydrolysis, have been obtained 3-amino-3-deoxy-D-glucose (LXV) (*50a*) and 6-amino-6-deoxy-D-glucose (kanosamine) (LXVI) (*51*). D-Talosamine (LXIX) is believed to form a component of cartilage (*52*), and D-fucosamine (LXX) (*52a*), along with glucose, galactose, and glucosamine, has been isolated from hydrolyzates of *Chromobacterium violaceum*. D-Mannosamine (LXVII) has been obtained from neuraminic acid (*52b*).



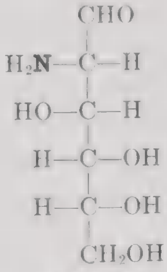
(LXIV)



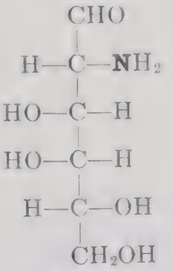
(LXV)



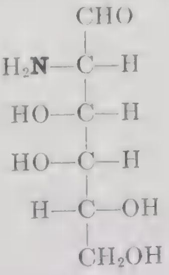
(LXVI)



(LXVII)



(LXVIII)



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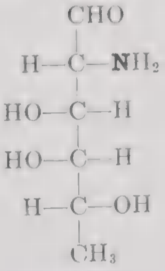


(LXX)

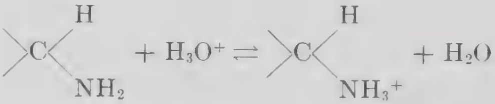
The combined amino sugars are usually found with their amino group acylated. In a few instances, e.g., in heparin the amino group is believed to be substituted by sulfate to give a sulfamidate

$$\mathrm{H{-}\overset{|}{\underset{|}{C}}{-}NH\cdot SO_2O^-}$$

which bears some analogy to the phosphoamidate structure characteristic of the phosphagens

$$\mathrm{{-}\overset{|}{\underset{|}{C}}{-}NH\cdot PO\cdot O_2^{2-}}$$

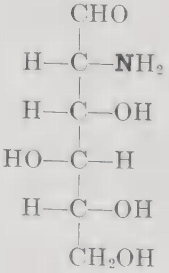
The free sugars, being primary amines, are readily protonated and form stable salts (e.g., Reaction 10).



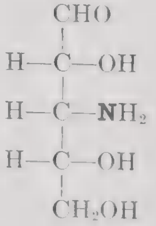
(10)

They are therefore considerably more resistant to decomposition by mineral acid than are the ordinary, nonamino sugars. Thus, many of the chemical reactions used to detect carbohydrates which depend on a transformation of monosaccharides into derivatives of furfural or 4-oxopentanoic acid (levulinic acid) and other substances (*53*), through the action of strong mineral acid, fail with the 2-aminohexoses (*53a*).*

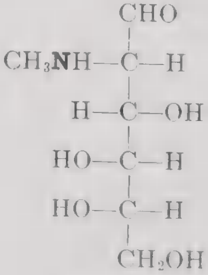
In recent years several interesting amino sugars, in addition to (LXV) and (LXVI), and including *N*-methyl derivatives, have been shown to occur in glycosidic combination as components of certain antibiotics. Streptothrycin and streptolin-B have been found to contain a moiety based on 2-amino-2-deoxy-D-gulose (LXXI) (*54*). 3-Amino-3-deoxy-D-ribose (LXXII) (*55*) has been isolated from the hydrolysis products of Achromycin (puromycin), and *N*-methyl-2-amino-2-deoxy-L-glucose (LXXIII) (*56*) is a component of streptomycin. Being a sec-



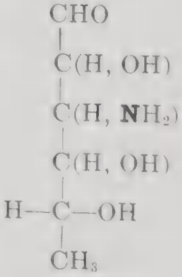
(LXXI)



(LXXII)



(LXXIII)



(LXXIV)

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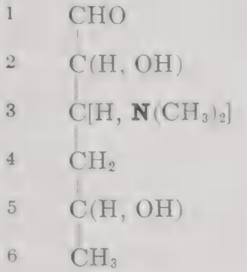
* It is important to note that amino sugars in which the amino group occupies positions 3, 4, or 6 do, in fact, give the Mölisch type of reaction whereas those having the nitrogenous substituent on C-2 do not [Whitehouse *et al.* (*53a*)].

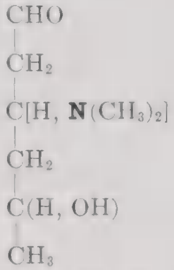
ondary amine, this sugar is more strongly basic than glucosamine itself. Mycosamine (LXXIV) (*57*), from Magnamycin, is a D-sugar; at the time of writing the stereochemistry has not been fully established.

Several dimethylamino sugars have been isolated from hydrolyzed antibiotics, but at the time of writing the stereochemistry of these substances also has not been fully established. Picromycin, several erythromycins, methmycin, neomethmycin, and novomycin all yield a dimethylamino-3,4,6-trideoxyhexose, variously termed picrocine (*58*), desosamine (*59*), and Amine-A (*60*), which has the general structure (LXXV):



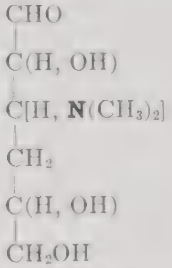
$$\begin{array}{ll} 1 & CHO \\ 2 & C(H, OH) \\ 3 & C[H, \mathbf{N}(CH_3)_2] \\ 4 & CH_2 \\ 5 & C(H, OH) \\ 6 & CH_3 \end{array}$$

(LXXV)



$$\begin{array}{l} CHO \\ CH_2 \\ C[H, \mathbf{N}(CH_3)_2] \\ CH_2 \\ C(H, OH) \\ CH_3 \end{array}$$

(LXXVI)

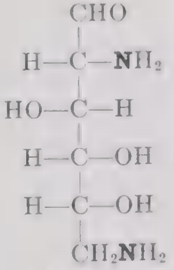


$$\begin{array}{l} CHO \\ C(H, OH) \\ C[H, \mathbf{N}(CH_3)_2] \\ CH_2 \\ C(H, OH) \\ CH_2OH \end{array}$$

(LXXVII)

A sugar, probably a stereoisomer of (LXXV) named mycaminose (*61*), has been obtained from Magnamycin (carbomycin); rhodosamine (LXXVI) (*62*), from rhodomycin, appears to be the 2-deoxy analog of the structure (LXXV). Amicetin yields amosamine (*63*) which is the 3,4-dideoxy analog of (LXXV), namely (LXXVII).

A newcomer to the sugar series is 2,6-diamino-2,6-dideoxy-D-glucose (LXXVIII) which is isolated, primarily as a disaccharide with D-ribose (XII), from neomycins B and C (*64*). (LXXVIII) has been named neosamine C. From *Bacillus subtilis* B also, a diaminohexose has been obtained (*65*).



$$\begin{array}{rcl} & CHO & \\ H- & C & -\mathbf{N}H_2 \\ HO- & C & -H \\ H- & C & -OH \\ H- & C & -OH \\ & CH_2\mathbf{N}H_2 & \end{array}$$

(LXXVIII)

V. Occurrence of Some Commoner Monosaccharides, Free and in Glycosidic Combination

In the free state, with the exception of D-glucose, and to a lesser extent, D-fructose, monosaccharides are normally found only in trace amounts in plant sap, in the tissue fluids and excreta of animals, and even in soils. These trace amounts must almost certainly be regarded as "escapes" from normal mechanisms of anabolism and catabolism. Nonetheless, the presence of such sugars does provide some confirmation of their participation in physiological metabolic pathways. The value of some of the older work on the assay of glucose (and other reducing sugars) in tissue fluids must be accepted with some caution since the methods used are not always entirely specific. It must be borne in mind, also, that preparations of glucose oxidase, which are of fungal origin, may contain glucosidases such as trehalase. In most organisms it seems that nearly all the carbohydrate of their tissues is present in combined states. An interesting survey of fungal polysaccharides has been made (*65a*).

A. D-GLUCOSE

1. *Free*

This sugar is, of course, a characteristic component of the blood and tissue fluids of vertebrate animals and has also been found in the tissue fluids of many kinds of plant. In a number of invertebrates—limpets (*66*), snails (*66*, *66a*), nematodes (*67*), and insects (*68*)—little or no free glucose can be detected. In arthropods—crabs (*66a*, *68a*) and crayfishes (*68b*)—and in species of *Arenicola* and *Sipunculus* D-glucose has been detected, by reliable procedures, in the body fluids (*68a*, *68b*). In locusts (*69*) and in some other insects (*68*) the nonreducing disaccharide trehalose (Section VI,A) seems to be the major carbohydrate component of low molecular weight.

In vertebrate blood, species differences occur with respect to the relative distribution of D-glucose between the water of the plasma and the water of the erythrocytes (*70*). Man is at one end of the scale, with approximately equal concentrations of the sugar in both erythrocytes and plasma, whereas the domestic fowl carries virtually all its blood glucose in the plasma (*71*). The hen's egg (*72*) and its 11-day embryonic allantoic fluid (*73*) both contain a little D-glucose.

2. *Combined*

Among oligosaccharides, polysaccharides (*74*), and natural glycosides (*74a*),[*] examples containing moieties of D-glucose are too numerous to mention. There are, however, one or two instances of somewhat unusual character which may be brought to the reader's notice.

Nervous tissue of vertebrates normally contains certain mixed, lipid-like, D-galactopyranosides and some D-glucopyranosides, having complex aglycones, and which are termed cerebrosides and gangliosides (*74b*). In the familial syndrome of Gaucher's disease, excessive amounts of these complex lipids are laid down in many tissues including the spleen. It is thought that such abnormally occurring cerebrosides contain unusual amounts of the D-glucopyranosyl derivatives (*75*). *O*-D-Glucopyranosides of mannitol, where the latter is substituted at position 1 or positions 1 and 6, have been isolated from *Laminaria cloustoni* (*75a*), from *Fucus spiralis* and *Desmarestia aculeata* (*75b*), and from the green algae *Enteromorpha compressa* and *Chlorella* sp. (*75c*).

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[*] The isolation of *O*-methyl-β-D-glucoside from *Scabiosa succisa* (mentioned in reference *74a*, p. 554) may be an artifact. (See reference *23*.)

The polysaccharide cellulose, so characteristic of the higher plants, has long been known to form the outer integument of the marine invertebrates known as tunicates. The chemistry of tunicate cellulose (*76*) and its physical nature are identical with those of the celluloses of angiosperms and gymnosperms and also with the extracellular cellulose produced by some bacteria (*77*). Recent observations, mainly based on X-ray crystallographic measurements have shown that human connective tissue appears to contain a small amount of material seemingly cellulosic in nature (*78*).

B. L-GLUCOSE

Two early reports (*79*) of the occurrence of this sugar have never been followed up nor confirmed.

C. D-FRUCTOSE

1. *Free*

Free D-fructose is widespread in plants; until comparatively recently it was considered to occur solely in the vegetable kingdom with the one exception that phosphoric esters,[*] which might yield "escape" amounts of fructose, were known to be of virtually universal occurrence. In 1907 Noel Paton *et al.* (*80*) reported that the embryonic fluids of certain ruminants gave color tests for hexuloses. This observation attracted little notice until 1948, when D-fructose was isolated from and identified in the blood of fetal sheep (*81*). It is now known that relatively large amounts of D-fructose are present in the blood of fetal ungulates [including the whale (*82*)] but that, in other species, e.g., man, only traces of this sugar occurs in the fetal circulation (*83*). D-Fructose has been identified in appreciable amounts also in the seminal plasma of numerous mammals where it is produced by organs accessory to the seminal tract (*84*). Birds (*85*) (domestic cocks) do not possess such accessory organs and their seminal plasma is probably devoid of detectable quantities of D-fructose, although it appears that traces may be formed by the spermatozoa themselves (*86*). Traces may be present in the hen's egg (*72*).

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[*] See Vol. I, Chapter 4, and Vol. II, Chapter 1.

The sugar has been identified also as the sole reducing carbohydrate in the larva of the fly (*Gastrophilus*) (*87*). D-Fructose is found in the urine of fructosurics (*88*), in which its presence may be due to a low kidney threshold or possibly excessive dephosphorylation.

2. *Combined*

Combined D-fructose is widespread in the higher plants. In these the fructosyl radicals are always in the furanose form; in sucrose, the oligosaccharides (see Section VI) derived from sucrose, and the storage polysaccharides (*89*) of the inulin (Compositae), levan (Gramineae), and irisin (certain but not all *Iris* spp.) or triticin (*Triticum* spp.) types. "Conventional" glycosides of D-fructose (that is, ones with a noncarbohydrate aglycone) are unknown to the writer; indeed the only such derivative of a hexulose is the adenylpsicofuranoside synthesized by *Streptomyces hygroscopicus* (*49*). There is no knowledge of any glycosidic compound of fructose being synthesized in the animal kingdom with the exception of sucrose, which appears sometimes to be of endogenous origin in the sucrosuric.*

D. D-GALACTOSE

1. *Free*

Apart from trace amounts D-galactose has been found free, in quantity, in a single but well-substantiated instance in the plant kingdom, namely as a crystalline exudate on ivy leaves after a severe frost (*89*). In the familial human syndrome of galactosemia the sugar is present in appreciable amounts in the blood and from thence is excreted by the kidney with the urine (*90*). Galactose and xylose are cataractogenic sugars (*91*); the galactosemic infant will develop cataract, and mental defect, if sources of dietary galactose such as lactose are not withheld.

2. *Combined*

In glycosidic combination, D-galactose is almost universally distributed. Although it is present in but a few of the known glycosides of the "conventional" type (*91a*), the sugar has recently been discovered in a variety of situations in combination with *myo*-inositol (*92*) and with glycerol (*93*) to form *O*-D-galactopyranosides. The former substance has been isolated from sugar beet (*92*), and the latter occurs in the marine algae *Irideae laminaroides* (*93*), *Gelidium pristoides* (*94*), and *Gracilaria confervoides* (*94*); it has recently been found in wheat flour (*95*). The oligosaccharides of milk (Section VI,B) contain D-galactose.

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* See reference *220*.

Polysaccharides (*96*) composed wholly or partly of D-galactose units are numerous. These include hemicelluloses, plant gums, and mucilages (*97*), algal polysaccharides (*98*), and some elaborated by bacteria and molds (*96*), a galactan of mammalian (beef) lung (*99*), frog spawn (*100*), and the galactan (galactogen) elaborated by the "albumin gland" of the vineyard snail (*Helix pomatia*), in which it is accompanied by L-galactose in the proportion of 1–6 moles (*101*). It is not known whether the L-sugar occurs in the "albumin gland" secretion of other species of *Helix*, but four species of aquatic snails have been examined and found to possess galactose-containing polysaccharides in which fucose and other sugars are present (*101a*).

The constituents of vertebrate nervous tissue, the gangliosides and cerebrosides (*75*) already mentioned, contain D-galactose. Most glycoproteins (*102*), that is, proteins containing a carbohydrate-derived moiety, almost invariably contain some galactose; very few proteins, as we know them, are completely devoid of carbohydrate. Indeed, the well-known Mölisch reaction for carbohydrates was originally devised as a test for proteins. Galactose is found in blood plasma proteins, mucous secretions, egg proteins (*103*), and the blood-group substances (*104*); in all these, other sugars occur as well, glucose among the common monosaccharides being absent. Microorganisms of many types elaborate macromolecules in which D-galactose (*105*)* is a major or a minor component.

3. *Combined* D-*Galactofuranose*

Penicillium charlesii G. Smith produces in its culture media a galactan which is unique in that it is composed of D-galactofuranose units (*106*). The D-arabitol-*O*-D-galactoside, umbilicin, isolated from lichens of the Order Gymnocarpeae, e.g., *Umbilicaria pustulata*, likewise has its sugar moiety in the furanose form (*106a*). Certain of the units of the polysaccharide of *Cladophora rupestris* are derived from D-galactofuranose (*106b*).

E. L-GALACTOSE

The sugar is unknown in the uncombined condition. Besides being present in *Helix pomatia* galactogen (see above) and in the jelly coat of eggs of *Echinus esculentus* (*106c*) it has been obtained from algal polysaccharides found in *Gelidium* spp. (agar) (*107*), *Chondrus ocellatus* (*107a*), *Chondrus crispus*, and *Gigartina stellata* (*109*) (Irish moss, carrageenin) (*108*), in *Porphyra crispata* (*109*), *P. tenera* (*109*), *P. capensis* (*110*), and *Gliopeltis furcata* (*110a*). Flaxseed mucilage (*111, 112*) also yields some L-galactose.

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* *Penicillium varians* makes a heteropolysaccharide in which the unit chains contain D-glucose at the nonreducing end; the reducing end is terminated by D-idose or D-altrose (*105*).

It may be recalled that the well-known mucic acid "test for galactose" does not distinguish between the D- and L-isomers (*113*).

F. D-MANNOSE

1. *Free*

The free sugar has been detected in small amounts in sphagnum mosses and in peat (*113a*).

2. *Combined* (*114*)

D-Mannose is found in the hemicelluloses of woods, especially from the gymnosperms and in the endosperms of certain seeds, especially nuts. It forms a major constituent of the cell wall of bakers' yeasts (*115*), along with the yeast glucan; and D-mannose-derived polysaccharides are found in a marine alga (*116*) *Porphyra umbilicalis* and in culture media of *Penicillium charlesii* (*117*). Small amounts of units derived from D-mannose have recently been found in samples of laminarin (*117a*). Along with other sugars, combined D-mannose is found in a few plant gums (*97*) and in *Mycobacterium tuberculosis* (*118*). Mannans are formed by *B. polymyxa* (*118a*) and diphtheria bacteria (*118b*).

D-Mannose forms a prominent part of the carbohydrate fragments of many glycoproteins including those in mammalian blood plasma (*102*) and hens' eggs (*103*).

Few if any terrestrial plant glycosides contain D-mannose, but a D-mannoside of D-glyceric acid occurs in algae of *Polysiphonia* spp. (*119*). A dimannoside of phosphatidylinositol has been found in a *Mycobacterium* (*120*).

G. D-XYLOSE

1. *Free*

D-Xylose is a sugar apparently missing from the animal kingdom[*] although it is not metabolically inert in mammals (*121, 122*); in its glycosidic derivatives it is largely confined to the higher plants. In extracts of the latter trace amounts of the free sugar have been found.

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[*] D-Xylose has recently been obtained from an invertebrate animal product (see reference *26a*).

2. *Combined*

Xylans (*123*), which may be composed entirely of D-xylose or which may contain minor amounts of other sugars, are plentiful in the woody tissues of all kinds of higher plants. The marine alga *Rhodymenia palmata* (*124, 125*) contains a xylan in which the units are arranged somewhat differently from those in the polysaccharides of terrestrial origin.

Plant gums from *Astragalus* spp. (genus Leguminosae) (gum tragacanth) (*126*); from wheat grain (*127*); from members of the Rosaceae, peach (*128*), almond (*129*), egg plum (*130*), and cherry (*131*); from the cactus *Opuntia fulgida* (cholla gum) (*132*); and from the leaves of *Phormium tenax* (New Zealand flax) (*133*)—all have been shown to contain moieties of D-xylose. Damson gum contains a much smaller proportion (*134*). These gums (*135*), which are exudates from the surfaces of the plant, may possibly be pathological manifestations and may vary in composition in a single plant (*136*).

From a number of different higher plants mucilages have been obtained that contain a proportion of D-xylose. Among *Plantago* spp. xylose-containing mucilages are found in *P. lanceolata* (*137*), *P. arenaria* (*138*), *P. ovata* (*139*), and *P. fastigiata* (*140*). Mucilages from quince seed (*141*), flaxseed (linseed) (*142*), and seeds from *Tamarindus indica* (*143*) also yield some D-xylose on hydrolysis.

In contrast with its prevalence in the higher plants, D-xylose is rare as a component of simple glycosides. The disaccharide primeverose, Xβ6G, glycosidically bound to alizarin, seems to be the sole example; it occurs in *Primula officinalis* (*145*), in madder *Rubia tinctorum* (*146*). Primeverose has also been found in the carob bean (*Ceratonia siliqua*) (*147*). Many years ago a nonreducing D-glucosyl-D-xyloside was described as occurring (as a dibenzoate) in the leaves of *Daviesia latifolia* (*148*). The exact structure of this substance does not appear to have been established.

The only example of a D-xylose-containing substance elaborated by a microorganism is afforded by the capsular slime of the human pathogen *Cryptococcus neoformans* (formerly *Torulopsis histolyticum*) (*149*). This ill-defined material contains also D-mannose, D-galactose, and a uronic acid.

H. L-FUCOSE

In combination, this sugar occurs in a wide variety of situations. Members of the Phaeophyceae, e.g., *Fucus, Laminaria, Ascophyllum,* *Pelvetia*, and *Macrocystis*, all yield an acidic polysaccharide, fucoidin, where the majority of the L-fucose units are esterified by sulfate (*150*). The sugar is frequently a component of the carbohydrate moieties of glycoproteins (*102, 103*), blood group substances (*104*) and also occurs in the oligosaccharides of human milk (Section VI,B). It has been found free (*104a*).

I. L-ARABINOSE

1. *Free*

Traces of the free sugar have been found in numerous situations (cf. Section VII).

2. *Combined* L-*Arabofuranose*

Many polysaccharides of vegetable origin are partly or wholly composed of L-arabofuranose units. Arabans occur in crude "pectin" (*151*) preparations from sugar beet (*152*), peanuts (*Arachis hypogaea*) (*153*), and apples (*154*). Many xylans (see Chapter 8, this volume) contain varying amounts of L-arabofuranose units attached to the main D-xylose chains; gums (*135, 136*) are also partly composed of this sugar. The blue-green alga *Anabaena cylindrica* (*154*) and the green alga *Cladophora rupestris* also contain combined L-arabinose.

3. *Combined* L-*Arabopyranose*

L-Arabopyranose units have been detected as components of gums from lemon, peach, cherry, and apple trees (*155*), from *Virgilia* (*155a*), and from *Acacia karroo* (*155b*). In these gums the great majority of the arabinose units have the furanose configuration. The disaccharide vicianose (Section VI,B,2) also contains an L-arabopyranose unit.

J. D-ARABINOSE

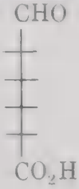
The sugar has been found only combined in bacterial products (*48a, 48b*) and in the Caribbean sponge (*48*).

K. L-RHAMNOSE

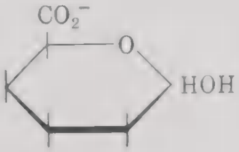
L-Rhamnose is very widely distributed among plants and is found in some bacterial products; in all these, isolated units derived from the sugar occur in gums (*97*), glycosides, and a number of polysaccharides of varying character. The sugar is not known to occur in animal tissues nor does it form homopolysaccharides in the manner of most other common sugars except D-ribose and 2-deoxy-D-ribose.

L. Uronic Acids

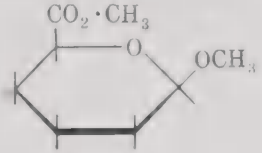
The uronic acids are acidic derivatives of monosaccharides in which the terminal carbon (bearing the highest number) is a carboxyl group; only six-carbon uronic acids ("hexuronic acids") have so far been found in nature. These free monosaccharide acids, like their parent hexoses, can form α- and β-glycosidic linkages and are, of course, "reducing" substances. In addition, being carboxylic acids, they can form anions, esters, etc. (LXXIX–LXXXI).



Aldehydohexuronic acid
(LXXIX)

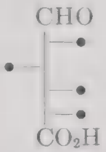


α/β-Hexopyranuronate ion
(LXXX)

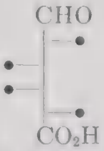


Methyl-O-β-hexo pyranuronoside methyl ester
(LXXXI)

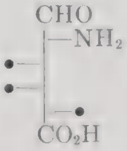
Six hexuronic acids are known in glycosidic combination (LXXXII–LXXXVII), one of these, D-galacturonic acid has been found free in detectable amounts in fruits (*156*).



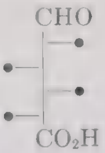
D-Glucuronic acid
(LXXXII)



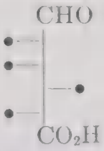
D-Galacturonic acid
(LXXXIII)



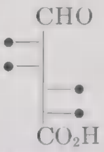
D-Galactosaminuronic acid
(LXXXIV)



L-Iduronic acid
(LXXXV)



L-Guluronic acid
(LXXXVI)



D-Mannuronic acid
(LXXXVII)

1. D-*Glucuronic Acid*

Derivatives of this acidic monosaccharide are widely distributed throughout the plant, animal, and microbiological kingdoms. In animals *O*-glycosidic derivatives of phenolic and other cyclic foreign chemicals are generated in the liver, kidney, and possibly other tissues (*156a*).

Heparin (*157*), the "mucoitin sulfuric acids" (*158*), and hyaluronic acid (*159*) are based on D-glucosamine and D-glucuronic acid in equimolar proportions. Many plant gums (*97*) contain D-glucuronic acid units. The acid has been isolated from hydrolysis products of polysaccharide material from brown algae where it occurs along with D-mannuronic and L-guluronic acids (*160*). Green-algal polysaccharides from *Cladophora rupestris* (*106b*), *Ulva lactuca* (*160a*), and *Acrosiphonia centralis* (*Spongomorpha arcta*) (*160b*) also yield D-glucuronic acid as do certain fungal products (*65a*) and certain bacterial polysaccharides, (Chapter 8), blood group substances, and chondroitin sulfate of animal connective tissue (*161*).

2. D-*Galacturonic Acid*

Like its glucose-derived isomer, D-galacturonic acid is found in many natural situations, where it enters into the composition of polysaccharides. No simple glycosides comparable with the animal glucuronosides are known.

D-Galacturonic acid forms the building material of the "pectic acid" fraction of pectin (*162*). A variable proportion of the carboxyl groups are in the form of methyl esters, and it is believed that some of the free hydroxyl groups are acetylated. With other sugars, it is found in certain gums (*97*); it does not appear to have been detected in fungal products (*65a*) although it comprises a major part of the capsular polysaccharide of *Pneumococcus* type I.

3. L-*Iduronic Acid and* L-*Guluronic Acid*

Improved methods of analytical separation have led to the discovery that L-iduronic acid units occur in "chondroitin sulfuric acid-B" (*163*) or β-heparin (*164*). L-Guluronic acid has been found to be a minor component of alginic acid (see below) (*160, 165*).

4. D-*Mannuronic Acid*

The acidic polysaccharide alginic acid, which is characteristic of brown algae, is unique in being composed virtually entirely of units of D-mannopyranuronic acid. Small amounts of L-guluronic acid are present in some samples (*165*). As far as the writer is aware no other occurrence of D-mannuronic acid is known.

5. D-*Galactosaminuronic Acid* (*165a*)

This substance is a component of the Vi-antigens of *Escherichia coli*, *Salmonella typhosa*, and *Paracolobactrum ballerup* and is the first natural aminohexuronic acid to be discovered.

M. Natural Compounds between Carbohydrates and Acids (Excluding Phosphoric)

In natural environments a number of mono- and disaccharides are known to occur with one or more of their hydroxyl groups esterified by carboxylic acid radicals. These radicals are acetyl, benzoyl, and trihydroxybenzoyl (galloyl). In addition pectic acid and gums are known to contain monosaccharide units which are acetylated, but, owing to the concomitant hydrolysis of the ester and glycosidic links, it has not been possible to determine the position of these ester groupings in the parent material. Birch wood xylan, which carries *O*-acetyl substituents has, however, been investigated (*166*).[*] 2-Carboxyacetyl (malonyl) and aminoformyl (carbamoyl) radicals are known to esterify carbohydrate moieties in certain fungal products. The free carboxyl group in the hydrogen malonyl esters confers acidic properties on the parent substances. (See also Section V,P.)

1. *Hydrogen Sulfates*

Hydrogen sulfate derivatives of polysaccharides are widespread. In some of these the esterified positions have been established, but no simple, or relatively simple, sulfated carbohydrates have been so far detected in a biological environment except the cerebroside sulfuric ester of beef brain, which is essentially a glycoside of D-galactose-6-*O*-sulfate (*166a*). Table I summarizes those monosaccharide units known to bear acid sulfate groups.

TABLE I

Sulfated Polysaccharide Types

Main units	Name of polysaccharide	Source	References
D-Glucose	(?) Cellulose sulfate	*Charonia lampas*[a]	(*167*)
D-Glucose	(?) Cellulose sulfate	*Busycon canaliculatum*[a]	(*168*)
L-Fucose, D-galactose, D-mannose, D-galacturonic acid, D-glucosamine	Limacoitin sulfate	Japanese snail	(*169*)
D- and L-galactose	Carrageenins	Red algae	(*170, 171*)
L-Fucose	Fucoidins	Brown algae	(*170, 171*)
D-Glucuronic and D-galacturonic acids D-Glucosamine and D-galactosamine	Heparin Keratosulfate Chondroitin sulfates	Animal connective tissues	
	Mactins	Clams	(*172*)

[a] Marine snails.

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[*] The acetyl groups are believed to occupy position 3 on the xylose units (*166*).

2. *6-O-Acetyl-D-glucose*

A cobalamine-producing strain of *Bacillus megatherium* (NCIB 8508) has been found to elaborate this glucose ester, which accumulates in the culture medium. Of fourteen strains of the microorganism which were examined, only three yielded detectable amounts of 6-*O*-acetyl-D-glucose (*173*). Although this article is not concerned with metabolic processes it is worth recording that experiments have been done on the effect of 6-*O*-acetyl-D-glucose on the reducing sugar level of the blood of the alligator (*174*).

3. *6-O-Benzoyl-D-glucose*

This substance, known as vaccinin, is found in the juice of blueberries (*175*) (*Vaccinium visitidaea L.*). 6-*O*-Benzoyl-D-glucose also occurs in combination with salicyl alcohol as a phenolic glycoside, populin (*176*), in members of the poplar (*Populus*) family.

4. *Dibenzoylglucosylxylose*

This substance was isolated from *Daviesia latifolia* (*148*); its structure has not been established.

A number of natural carboxylic acid glycosidic derivatives of monosaccharides have been found. In these substances the acid radical plays the part of the aglycone.

5. *β-D-Glucuronosidyl benzoate* (*177*)

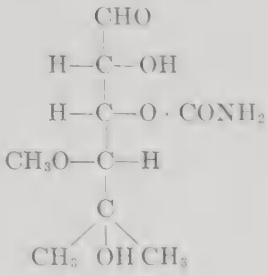
One pathway by which aromatic acids are eliminated from the vertebrate body involves "conjugation" with D-glucuronic acid through glycoside formation. It is not known how far down the evolutionary scale this kind of reaction may be found. At present, compounds of this type appear to be confined to mammals and birds (*177a*).

6. *β-D-Glucosyl Benzoate* (*178*) and *β-D-Glucosyl Galloate* (*179*)

The glucose analog of the substance mentioned above has recently been isolated from the laterocervical organs of *Periplaneta americana* L. and *Blatta orientalis* L. This animal product is comparable with the galloate (2,4,6-trihydroxy benzoate) glucogallin found in Chinese rhubarb. The biological role of these substances is unknown.

7. *3-O-Carbamoylnoviose* (*45*)

The antibiotic novobiocin contains units derived from the 3-*O*-carbamate (LXXXVIII) of noviose (Section I,E):



(LXXXVIII)

8. *Isovalerylmycarose* (*44*)

The 2,6-Dideoxy-3-*C*-methylhexose, mycarose (Section I,E), units of which are contained in the antibiotic Magnamycin, has one of its hydroxyl groups esterified by isovaleric acid.

9. *Hydrogen Malonyl Esters in Mold Polysaccharides*

Penicillium luteum Zukal elaborates an acidic polysaccharide, luteic acid, which accumulates in the culture medium. Complete hydrolysis of luteic acid yields D-glucose and malonic acid in equimolar proportions; saponification of the ester linkage gives the neutral glucan luteose (*181*). An analogous polysaccharide also based on D-glucose units, but bearing malonyl radicals in the ratio of 2:1, has been obtained from *Penicillium capreolinium* (*182*).

N. Natural Monosaccharides Bearing *O*-Methyl Groups

In Sections I,D and I,E a number of sugars concerned in the formation of steroid glycosides and antibiotics have been noted. The monosaccharides concerned are deoxy sugars. During the past few years the occurrence of *O*-methyl derivatives of common hexoses and of D-glucuronic acid has come to light. The origin of the methyl groups is not known. The 2-*O*-methyl ethers of fucose and rhamnose and the 2,4-di-*O*-methyl ether of rhamnose have been detected as components of glycolipids of *Mycobacterium* spp. (*182a*).

1. *4-O-Methyl-D-glucuronic Acid* (*183*)

Units of this uronic acid are widespread, e.g., in gums from lemon (*184*) mesquite (*185*) and myrrh (*186*); in woods from aspen (*187*), *Eucalyptus regnans* (*188*), white elm (*Ulmus americanus*) (*184, 189*), white birch (*Betula papyrifera*) (*190*), Sitka spruce (*191*). It has also been found in jute hemicellulose (*192*). The supposed "3-*O*-methyl glucuronic acid" of Das Gupta and Sarkar (*193*) is in fact methylated in the 4 position (*194*).

2. *3-O-Methyl-*D*-glucose*

This sugar, which was one of the first partially methylated glucoses ever to be prepared in the laboratory has been identified among the hydrolytic products of the venom of a holothurian (*26a*).

3. *3-O-Methyl-*D*-galactose*

Hydrolyzates of the nonacidic portion of the mucilage of *Ulmus fulva* contain 3-*O*-methyl-D-galactose (*194a*).

4. *6-O-Methyl-*D*-galactose*

Hydrolysis of polysaccharide material from the marine alga *Porphyra capensis* yields, among other D- and L-galactose derivatives, the 6-methyl ether (*110*).

5. *2-O-Methyl-*L*-fucose*

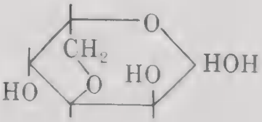
Recent work indicates that units derived from this ether of L-fucose are quite widespread in material of plant origin. It has been isolated from hemicelluloses from Victoria plum leaves (*195*) and from pectins of sugar beet (*195a*) and sisal (*195b*); evidence of its presence in the yew (*Taxus cuspidata*) has also been obtained.

6. *2-O-Methyl-*D*-xylose*

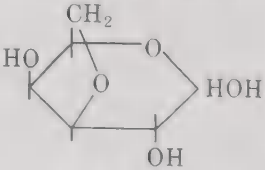
This sugar occurs, along with 2-*O*-methyl-L-fucose, in plum leaf hemicelluloses (*195*). Chromatographic evidence of its occurrence in soil has also been obtained (*196*). It is quite widespread in plant polysaccharides.

O. 3,6 ANHYDRO-L- AND -D-GALACTOSE UNITS IN ALGAL POLYSACCHARIDES

In 1938 Araki (*196*) and Hands and Peat (*197*) reported the detection among the hydrolysis products of agar (*Gelidium* spp.) of 3,6-anhydro-L-galactose (LXXXIX). Forbes and E. G. V. Percival (*198*) slightly later reported independent confirmation of the presence of this hitherto unique internal sugar ether in agar in which L-galactose itself had already been noted as a constituent. Araki and his co-workers have



α/β-3,6-Anhydro-L-galactopyranose
(LXXXIX)



α/β-3,6-Anhydro-D-galactopyranose
(XC)

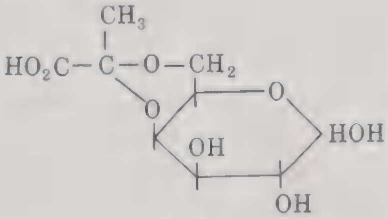
contributed much to our subsequent knowledge of the structure of agar, in numerous papers published in the *Bulletin of the Chemical Society of Japan* and elsewhere (*199*).

In 1954 O'Neill reported the isolation of a derivative of the optical isomer of the above substance, namely 3,6-anhydro-D-galactose (XC) from the *kappa*-carrageenin of *Chondrus crispus* (*200*) and E. E. Percival simultaneously isolated the same substance from *Chondrus* whole polysaccharide (*201*).

Whether the units derived from the 3,6-anhydro sugars are formed by the algae *in vivo*, or whether they arise during isolation through loss of sulfate ester groups with concomitant formation of the internal ether is not known. It is important to note that the 3,6-anhydrohexose gives some color tests normally attributable to hexuloses, e.g., the Selivanoff reaction.

Polysaccharides from other red algae, *Gliopeltis furcata* and *Chondrus ocellatus*, both contain 3,6-anhydro-L-galactose units (*107a*, *110a*).

P. Pyruvic Acid-Derived D-Galactose Cyclic Ketal Units in Agar



(XCI)

Units based on structure (XCI) have been shown to be present in agar (*202*). The structure of such a natural ketal, in this case derived from pyruvic acid, is so far unique.

VI. The Occurrence of Oligosaccharides

In the subsequent pages the following symbols will be used:

G	for	D-glucopyranose unit
GA	for	D-glucuronic acid unit
Ff	for	D-fructofuranose unit
Ga	for	D-galactopyranose unit
Gnac	for	*N*-acetyl-D-glucosamine unit
Rh	for	L-rhamnopyranose unit
Fuc	for	L-fucopyranose unit
A	for	L-arabinopyranose unit
X	for	D-xylose unit
∨	for	α-glycosidic linkage
∧	for	β-glycosidic linkage

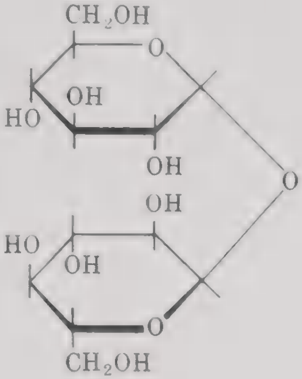
Taking the physiological point of view, di- and higher saccharides which are found in biological environments can be classified into four main groups. First we have those reducing substances that are found only in relatively small amounts because they occur as dynamic intermediaries in some anabolic or catabolic system such as starch-glucose from which maltose (G \/ 4G) and maltotriose (G \/ 4G \/ 4G) can be sometimes isolated. Secondly we find reducing oligosaccharides in nonreducing glycosidic combination with noncarbohydrate radicals (aglycones) (*203*) from which they may be liberated, with the intersugar links intact, by an appropriate hydrolytic procedure which may take place in the natural environment. An example is the disaccharide primeverose (X /\ 6G) which occurs naturally in glycosidic combination with alizarin as "ruberythric acid" (X /\ 6G—"alizarinyl"). Thirdly we have the unique group of reducing sugars which are excreted from the mammary glands of female mammals and comprises lactose (Ga /\ 4G) with smaller amounts of reducing tri-, tetra-, penta-, and hexasaccharides built up by attachment of various sugar units to lactose itself. These sugars do not appear to undergo any changes in the milk. As will be noted later, lactose, so commonly regarded as a sugar unique to the female mammal, is also to be found in the vegetable kingdom. Fourthly, we have the two widely distributed nonreducing disaccharides sucrose (G /\/ Ff) and trehalose (G \/\/ G) which seem to be forms in which potential energy can be transported or stored in a readily available form in the sap or body fluids of many organisms. In addition, it has become clear that sucrose molecules, and possibly trehalose as well, form "anchors" on to which may be attached considerable numbers of other sugar units, usually in chains, to generate higher oligo- and polysaccharides. The fructosans of the inulin and levan types can often be shown directly or indirectly to contain a moiety having the sucrose structure (see Chapter 8).

The nonreducing disaccharides and their homologs will be considered first.

A. Nonreducing Oligosaccharides

1. *αα-Trehalose*

This sugar (XCII) has been known for a very long time; it was first obtained from ergot in 1832 (*204*) but got its name from trehala manna (of insect origin) from which it was isolated in 1859 (*205*). A full historical account of early work on trehalose has been published (*206*). The sugar occurs in a wide variety of situations, in fungi, freshwater algae, lichens, a pteridophyte (*Selaginella lepidophylla*), yeasts,



$\alpha\alpha$-Trehalose

(XCII)

TABLE II
SOURCES OF $\alpha\alpha$-TREHALOSE

Group	Name of organism	State in which sugar was present *in vivo*	References
Insects	Locust	Free	(*69*)
	Silkworms (*Bombyx*)	Free	(*206a*)
	Various	Free	(*67*, *68*, *206b*)
	Cestodes and nematodes	Free	(*206c*)
Bacteria	*Propionobacterium* (3 spp.)	Free in cells and medium	(*207*)
	Mycobacterium tuberculosis	Esterified in neutral fat fraction	(*208*)
	Mycobacterium phlei	Free	(*209*)
	Mycobacterium tuberculosis	Esterified as 6,6′-dimycolate	(*210*)
Fungi	"Mushrooms"	Free	(*211*)
	Pholiota spectabilis ("big laughing toadstool")	Free	(*212*)
Lichens	*Umbilicaria pustulata*	Free	(*213*)
	Cladonia rangiferina, *Umbilicaria rigida*, *Haematomma ventosum*, *Cetraria islandica*, *Leconora arcta*	Free	(*214*)
Algae	Freshwater, Rhodophyta: *Lemanea nodosa* and *Sacheria fucina*	Free	(*214a*)
	Freshwater, Cyanophyta: *Phormidium tenere*	Free[a]	(*215*)
	Marine, Cyanophyta: various spp.	Free	(*215a*)
Yeast		6-Phosphate	(*216*)
	Bakers'	Free	(*217*)
	Brewers'	Free	(*218*)

[a] Circumstantial evidence.

TABLE III

THE SUCROSE FAMILY OF NATURAL OLIGOSACCHARIDES[a]

Formula	Trivial name	Occurrence	References
[Ff /\/ G6] \/ Ga	Raffinose	Widely in green plants	(*221*)
	6^{G}-α-galactosylsucrose	Birch (*Betula verrucosa*)	(*223*)
[Ff /\/ G6] \/ Ga \/ 6Ga	Stachyose	Widely in green plants including Leguminosae and Labiatae	(*221–223*)
		Birch	(*223*)
[Ff /\/ G6] \/ $(\mathrm{Ga6})_2$ \/ 6Ga	Verbascose	*Verbascum*, *Salvia*, *Ajuga*	(*222*, *224*)
[Ff /\/ G6] \/ $(\mathrm{Ga6})_3$ \/ 6Ga	Verbascotetraose		(*222*)
[Ff /\/ G6] \/ $(\mathrm{Ga6})_4$ \/ 6Ga	Verbascopentaose		(*222*)
Ga \/ [3Ff /\/ G]	Planteose 3^{F}-β-galactosyl sucrose	*Plantago* spp., *Fraxinus* manna, tobacco seeds (Solanaceae).	(*225*)
		Mint	(*225a*)
Ga \/ [1Ff /\/ G6] \/ Ga	Lychnose[b]	Caryophyllaceae	(*222*)
Ga \/ [3Ff /\/ G6] \/ Ga	Isolychnose[b]	Caryophyllaceae	(*222*)
G \/ [3Ff /\/ G6]	Melizitose 3^{F}-β-glucosyl sucrose	Exudates of animal origin found on plants, e.g. "mannas" and "honeydews"	(*226*)
[Ff /\/ G2] \/ Ga	2^{G}-α-galactosyl sucrose	Umbelliferae	(*227*)
Ff /\ [6Ff /\/ G]	Kestose 6^{F}-β-fructofuranosyl sucrose	Gramineae, e.g. *Lolium perenne* and Rye	(*228*) (*227a*)
		Honey	(*228a*)

Ff /\ [1Ff /\/G]	Isokestose 1^F-β-fructofuranosyl sucrose	*Allium* spp., *Lolium multiflorum*, *Arrhenatherum elatius* Rye *Campanula* sp.	(*229*) (*227a*) (*229a*)
[Ff /\/ G6] /\ Ff	Neokestose 6^G-β-fructofuranosyl sucrose	*Allium* spp., *Lolium multiflorum*, *Arrhenatherum elatius* Wheat	(*229*) (*227b*)
[Ff /\/ G6] /\ G	Gentianose 6^G-β-glucosyl sucrose	*Gentiana* spp.	—
[Ff /\/ G4] \/ G	"Maltosyl" fructoside 4^F-α-glucosyl sucrose	Aphid honeydew	(*230*)
Ff /\ [1Ff /\/ G] 6 /\ Ff	$1^F,6^F$-(di-β-fructofuranosyl) sucrose	Gramineae	(*227a*)

---

[a] All components belong to the D-family.

[b] Both lychnose and isolychnose are accompanied by higher homologs (*222*).

and bacteria. Recently trehalose has been demonstrated to be the main, if not the sole, carbohydrate of small molecular weight in insect hemolymph. So far it has not been found in higher plants (angiosperms) nor in vertebrates. Trehalose monophosphate has long been known to occur in yeasts.

Table II illustrates the diversity of sources from which αα-trehalose has been obtained in recent years.

2. *Sucrose and Oligosaccharides Derived from Sucrose*

Sucrose (G \/\ Ff) is probably universal in the photosynthetic plants in which it occurs in very variable amounts. It would appear that sucrose has two parts to play in the metabolism of these organisms; the first is to provide an easily translocated source of material from which free energy may be obtained or which may be converted into other simple sugars, and the second role is to provide a "starter molecule" to which may be attached units of glucose, galactose, or fructofuranose in increasing numbers until polysaccharides are ultimately formed. It seems possible that some of the oligosaccharides derived in this way from sucrose may be intermediates in polysaccharide synthesis. Oligosaccharides such as raffinose (galactosyl sucrose, G \/ 6G \/\ Ff) and its higher galactosylated homologs may act as a means of translocating galactosyl radicals.

There is considerable evidence that the fructofuranosans of plant origin, levans, inulins, and possibly also those of the irisin-triticin type are built up from a sucrose starter molecule, e.g.,

Ff . . . . . . Ff . . . . . . Ff \/\ G . . . . . . Ff . . . . . . Ff

or

Ff . . . . . . Ff . . . . . . Ff \/\ G

Many such polysaccharides have been shown to contain a glucose moiety (*219*); from some of them sucrose has actually been isolated after carefully controlled hydrolysis.

Sucrose is not commonly regarded as being a product of the animal kingdom. However, a very few human cases of endogenous sucrosuria have been reported (*220*). In these individuals, "endogenous sucrosurics," sucrose is excreted in the urine when the sugar is absent from the diet. It may be pointed out that animals almost certainly possess the materials out of which sucrose could be synthesized, namely D-glucosyl uridine diphosphate and fructofuranose molecules. A sucrose phosphate has been found as a product of photosynthesis (*220a*).

Oligosaccharides based on a sucrose moiety have so far been found to contain in addition units derived from only D-glucose or D-galactose. Table III indicates the structures and sources of these substances.

B. REDUCING OLIGOSACCHARIDES

1. *Lactose and Oligosaccharides Derived from Lactose*

Lactose was first discovered to be present in milk by Fabriccio Bartoletti in 1615 and, as has been remarked above, is frequently stated to be a product peculiar to lactating females of the mammalian class.

TABLE IV
LACTOSE-DERIVED OLIGOSACCHARIDES FROM HUMAN MILK (*235*, *235a*)

Structure	Trivial name
Ga /\ 4G	Lactose
[Ga /\ 4G][a] 2 \/ Fuc	Fucosyllactose Ga^2-α-L-fucosyl lactose
[Ga /\ 4G] 2 3 \/ \/ Fuc Fuc	Lactodifucotetraose Ga^2-G^3-(di-α-L-fucosyl lactose)
Ga /\ 3Gnac /\ [3Ga /\ 4G]	Lacto-*N*-tetraose
Ga /\ 3Gnac /\ [3Ga /\ 4G] 2 \/ Fuc	Lacto-*N*-pentaose I
Ga /\ 3Gnac /\ [3Ga /\ 4G] 4 \/ Fuc	Lacto-*N*-pentaose II
Ga /\ 3Gnac /\ [3Ga /\ 4G] 2 4 \/ \/ Fuc Fuc	Lacto-*N*-fucohexose I
Ga /\ 3Gnac /\ [3Ga /\ 4G] 4 3 \/ \/ Fuc Fuc	Lacto-*N*-fucohexaose II

[a] The lactose moiety is bracketed.

For example, as recently as 1934 it has been stated that lactose "has not been encountered in the plant kingdom" (*231*). Nonetheless, it was isolated in 1871 from ripe fruits of *Achras sapota* L. (*232*), in which its occurrence was adequately confirmed in 1958 (*233*). Its reported detection in flowers of *Forsythia* (*234*) has been disproved.

During the past few years the presence in human milk of a number of interesting oligosaccharides has come to light. These are either present only in trace amounts or absent from certain other milks, notably that of the cow, ewe, and goat. Nonruminant milks, of the cat, monkey, dog, rabbit, mare, and sow, are richer in these lactose derivatives. The original literature has been reviewed (*235*) and should be consulted for details of this interesting collaboration between pediatricians in the United States and chemists in Germany. The human milk oligosaccharides, which contain units derived from L-fucose and *N*-acetyl-D-glucosamine, are listed in Table IV.

Free lactose is found in the urine of lactating women (*235a*); as far as other mammals are concerned, the writer can find references only to the lactating cow (*235b*). It would be interesting to know whether the unfortunate condition of gynecomastia in men is accompanied by lactosuria.

2. *Other Reducing Oligosaccharides*

In this section consideration will be confined to substances other than lactose and its derivatives, which have been definitely identified in tissues or products of biological origin. A number of di- and higher saccharides have been found in small amounts; as has been already stated these substances are obviously metabolic intermediates in the hydrolytic fusion of larger molecules or in the anabolic production of more complicated carbohydrates. Little is known of the existence of these processes although a few enzymes with transglycosylating activity have been found, for example, the plant "D-enzyme" of Rees, the bacterial "amylomaltase" of Monod and Torriani, and the rat liver enzyme of M. R. Stetten (*235c*).

The remainder of the reducing saccharides to be described in this section are derived from "typical glycosides." A number of di- and higher saccharides occur, chiefly in steroid glycosides, in which the modes of combination of the monosaccharide units are not yet established; these substances are therefore omitted. Di- and higher saccharides obtained synthetically or by controlled hydrolysis of polysaccharides are likewise not described.

Table V summarizes the main bulk of the natural oligosaccharides.

TABLE V
REDUCING DI- AND HIGHER SACCHARIDES OF PHYSIOLOGICAL ORIGIN

Structure	Trivial name	Source	References
G \/ 2G	Kojibiose	*Aspergillus oryzae* fermentation	*(236)*
G \/ 3G	Nigerose	Beer	*(236a)*
G \/ 4G	Maltose	Rat diaphragm muscle	*(237)*
		Rat liver	*(238)*
		Honeydew on *Trifolium hybridum*	*(239)*
		Many plant extracts	—
G \/ 4G \/ 4G	Maltotriose	Rat diaphragm	*(237)*
		Rat liver	*(238)*
		Plant extracts	—
		Chlorella	*(238)*
G \/ 4G \/ 4G \/ 4G	Maltotetraose	As for maltotriose	—
G /\ 2G	Sophorose	Plant glycosides[a]	*(203)*
		Sophora japonica L.[a]	*(240)*
		Stevia rebaudiana[a]	*(241)*
G /\ 3G	Laminaribiose	Needles of *Pinus sylvestris*	*(242)*
G /\ 4G	Cellobiose	Glycolipid of *Ustilago zeae*[a,b]	*(242a)*
G /\ 6G	Gentiobiose	*Gentiana* spp.[a]	*(203)*
X /\ 6G	Primeverose	Plant glycosides[a]	*(203)*
		Macrozamin from *Macrozamia spiralis* and *M. riedlei*	*(242b)*
A /\ 6G	Vicianose	Plant glycosides[a]	*(203)*
GA — 2GA	—	Glycyrrhinic acid[a,b]	*(242c)*
Ga \/ 6G	Melibiose	Traces in honey, honeydew	
Ga \/ 6Ga \/ 6G	Manninotriose	Accompanies stachyose, etc. (q.v.)	
G /\ 3Ga	Solatriose	α-Solanin (*Solanum* spp.)[a]	*(243)*
G /\ 3Ga 2 \/ Rh	Solatriose	α-Solanin (*Solanum* spp.)[a]	*(243)*
G /\ 3Ga	Solabiose	β-Solanin (*Solanum* spp.)[a]	*(243)*
G /\ 2G /\ 4Ga 3 /\ X	Lycotetraose	α-Tomatin[a]	*(244)*
Rh /\ 6G	Rutinose	Plant glycosides[a]	*(203, 245)*

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[a] The sugar is found in glycosidic combination.

[b] The sugar component has been proved to have the structure indicated but has not so far been isolated in the unhydrolyzed state.

VII. Chromatographic Examinations of Biological Materials for Free Sugars

A wide range of biological materials have been examined, largely by chromatographic methods, with the result that the presence in them of many free sugars has been detected. In the appended list, which does not claim to be in any way complete, the sugars detected have not been, on the whole, completely characterized. As has been stated earlier, chromatography does not distinguish between D- and L-isomers; in addition there is always the possibility of more than one sugar migrating at the same rate. In experiments which led to the discovery of D-apiose among the sugars obtained, mild acid treatment of tissues of *Posidonia australis* (*24*) it was found that the sugar in question could frequently behave chromatographically as if it were rhamnose.

The materials reported are listed in a rough alphabetical order.

List of Materials Reported

Aconitum napellus L. (*246*)
Alfalfa (*Medicago sativa*) (*247*)
Allium spp. (*229, 248, 249*)
"Aphids": Woolly alder (*250*)
    Coccus hesperidium (*251*)
    Eucallipterus tiliae L (*226*)
    Tuberolachnus salignus (*251a*)
Artichoke (*Helianthus tuberosus*) (*252*)

Cereals (*253*)
Chlorophyta (*254*)
Clover (*Trifolium pratense*) (*255*)
Collagen hydrolyzates (26 vertebrate and invertebrate spp.) (*256*)
Crassulaceae spp. (*257*)

Eugenia jambolana (*258*)

Flowers: Tea (*262*)
Fruits: Apple, plum, pear (*259*)
    Apricot (*260*)
    Banana (*261*)

Gramineae: Barley (*264*)
    Lolium sp. (*228*)
    Lolium sp. and *Arrhenatherum* sp. (*229*)
    Lolium sp. and *Festuca* sp. (*263*)

Heather (*Calluna vulgaris*) (*265*)
Honey (*228a, 266, 273a*)
Hop cones (*Humulus* sp.) (*267*)

Liposaccharides of *Pseudomonas aeruginosa* (*269*)
Liver extracts (*268*)
Lycoris radiata bulbs (*270*)

Maple (*Acer saccharum*): Sap (*271*)
    Syrup (*272*)

Nectar (*273, 273a*)

Palm (*274*)
Parsnip root (*275*)
Peat (*113a, 265*)
Placenta (human) (*276*)
Pollen: *Cryptomeria japonica* (*278*)
    Pinus montana (*277*)
Poppy (*280*)
Potato (*279*)
Primula elatior (*281*)

Saxifragaceae (*281a*)
Seeds: Barley (*281b*)
    Beans: Caracas cacao (*282*)
        Carob (*Ceratonia siliqua*) (*147*)
        Coffee (fermenting) (*283*)
        Lima (*284*)
        (*Vicia faba*) (*224*)
    Gramineae (*285*)
    Maize (*286*)
    Nuts and almonds (*287*)
    Nuts, hazel, Turkish (*Corylus* sp.) (*288*)
    Rubber (*Hevea brasiliensis*) (*289*)
    Tea (*290*)
Seminal plasma, various mammals (*290a*)
Soils (*291*)
Sphagnum (*113a, 265*)

Tobacco (*292*)
Tomato (*293*)

Urine (human, normal and muscular dystrophic on normal diets) (*294*)[a]

Wheat flour (*295*)
Woods: Birch (*Betula verrucosa*) (*223*)
    Cedar (*Chamaecyparis lawsoniana*) (*296*)
    Cedar, Western (*Thuja plicata*) (*297*)
    Pinus sp. (*298*)

Yolk, egg, lipoprotein (*299*)

[a] The following sugars were detected by White and Hess (*294*): arabinose, xylose, xylulose, ribose, ribulose, glucose, fructose, galactose, fucose, sedoheptulose, mannoheptulose, lactose, and sucrose.

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CHAPTER 8

Polysaccharidases

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I. Introduction

Polysaccharidases are defined as the enzymes that catalyze the hydrolytic breakdown of polysaccharides into products of lower molecular weight yielding, after exhaustive action, usually mono-, di-, tri-, or tetrasaccharides and frequently oligosaccharides or other polysaccharides of various molecular weights. These enzymes are highly specific for their respective substrates with regard to the chemical nature of the monomeric sugar units involved, as well as with respect to chemical and optical configurations of the glycosidic linkages to be hydrolyzed. The number and variety of polysaccharidases would be expected, therefore, to equal or even to exceed the number and variety of their substrates, polysaccharides.

TABLE I
CLASSIFICATION BY COMPOSITION AND STRUCTURE OF POLYSACCHARIDES KNOWN TO BE SUBSTRATES OF HYDROLYTIC ENZYMES

- **I.** Polysaccharides containing no amino sugars
  - *A.* Homoglycans
    - *1.* Polyglucosans
      - *a.* Amylose, amylopectin, glycogen
      - *b.* Dextran
      - *c.* Cellulose
      - *d.* Lichenin
      - *e.* Laminarin
    - *2.* Polymannans and polygalactans
    - *3.* Polyfructans
      - *a.* Glucofructan
      - *b.* Inulin
    - *4.* Polypentosans
    - *5.* Polyuronides
      - *a.* Pectic substances
      - *b.* Alginin
      - *c.* Hemicellulose
  - *B.* Heteroglycans (most bacterial polysaccharides)
- **II.** Mucopolysaccharides (*1*)
  - *A.* Neutral mucopolysaccharides
      - *a.* Chitin
  - *B.* Acid mucopolysaccharides
    - *1.* Simple acid mucopolysaccharides
      - *a.* Hyaluronic acid
    - *2.* Sulfomucopolysaccharides
      - *a.* Heparin
      - *b.* The chondroitin sulfates
    - *3.* Phosphomucopolysaccharides
      - *a.* Substrate of lysozyme
  - *C.* Mucoproteins + mucoids

The classification of polysaccharidases as to enzymatic specificity would then be analogous to the classification of polysaccharides according to their chemical composition and structure (see Table I).

In spite of the tremendous number of theoretically possible polysaccharide hydrolyzing enzymes, a relatively small number of such enzymes has been investigated so far and sufficiently characterized to warrant their discussion for the purpose of a comparative treatise. A large number of polysaccharidases has been detected only in lower plant life; i.e., in bacteria, molds, or yeasts and there, frequently only in one or two particular organisms.

Enzymes like maltase, cellobiase, and saccharase will not be included in the present discussion since their respective substrates are disaccharides rather than polysaccharides. Furthermore, enzymes that catalyze the synthesis as well as the degradation of polysaccharides through nonhydrolytic transfer reactions do not belong to the class of hydrolases and will not be discussed, therefore, with the polysaccharidases.

II. The Enzymes of Glycogen and Starch Degradation

Among all polysaccharidases, greatest interest has been attracted by the enzymes digesting starch and glycogen because of the widespread occurrence of their substrates in nature and because of the obvious and marked effect produced by their action. Thus, as early as 1811, Kirchhoff (*2*) reported the digestive action of wheat extracts upon starch, which probably constitutes the first description of an enzymatic process. Similar phenomena were soon described in other sources, namely, in saliva by Leuchs (*3*), in malt by Payen and Persoz (*4*), and in pancreas by Bouchardat (*5*). The active principle of saliva was termed ptyalin by Berzelius, and that of malt was called diastase by Payen and Persoz. Later, it was suggested by Beijerinck (*6*) to term all starch-digesting enzymes amylases. Such enzymes have subsequently been found in a large variety of other sources, notably in blood and urine, in numerous higher plants, and in a great number of different microorganisms.

From our present knowledge of the chemical structure of starch and glycogen and of the mechanism of their enzymatic digestion we can recognize at least six distinct classes of enzymes causing degradation of these substances.

a. Hydrolytic enzymes which split α-1,4-glucosidic linkages and produce mainly maltose, maltotriose, higher molecular weight oligosaccharides and dextrins, but only insignificant amounts of glucose. These enzymes are α-amylases and β-amylases.

b. Hydrolytic enzymes which split α-1,4-glucosidic linkages and liberate glucose either as the sole product of reaction or in conjunction with dextrins. To this class belong amyloglucosidases and glucamylase.

c. Hydrolytic enzymes which split α-1,6-glucosidic linkages in glycogen and amylopectin. These are amylo-1,6-glucosidase and R-enzyme.

d. Hydrolytic enzymes splitting both α-1,4- and α-1,6-glucosidic linkages, usually just termed α-glucosidases, or limit dextrinase, and *Clostridium* maltase.

e. Enzymes of presumably hydrolytic character, the exact nature of the linkages split by them being unknown, however, e.g., Z-enzyme (*7*).

f. Nonhydrolytic enzymes which catalyze both the synthesis and degradation of the polysaccharides in a reversible manner, such as phosphorylases and transglucosidases, including Q-enzyme, amylomaltase, and the so-called "amylase" of *Bacillus macerans.*

Although the enzymes of the latter class are able to bring about the degradation of starch or glycogen under certain circumstances, they do not belong to the polysaccharidases since they are not hydrolases, and they will not be included, therefore, in the present discussion. Names like amylomaltase, amylosucrase, and, in particular, amylase of *B. macerans* may tend to produce confusion between these enzymes and the true amylases. For this reason, the name of cyclodextrinase had been suggested earlier for the "amylase" of *B. macerans* (*8*). Furthermore, Z-enzyme will not be discussed because of its controversial nature (*9*) and because of the uncertainty of the chemical nature of the glucosidic bond it is believed to hydrolyze.

A. α-AMYLASES

1. *Changes in the Properties of the Substrate Caused by Action of Enzyme*

Although the picture of the phenomena noticeable during α-amylase action may be blurred by the presence of other amylolytic enzymes, the action of pure α-amylase is characterized by the concurrent appearance of the following manifestations, independently of the source from which the α-amylase was obtained.

a. Increase in Reducing Power of the Substrate. This increase is due to a decrease in molecular weight of the substrate (amylose, amylopectin or glycogen solutions, or starch paste) resulting in an increase of both reducing and nonreducing end groups. The reducing power can be measured by any of the commonly used methods for aldehyde determinations, e.g., by the copper reagent of Somogyi (*10*). Since the reducing sugars in an α-amylatic digest are mixtures of oligosaccharides with widely varying chain length, the reducing values obtained generally do not reflect an exact picture of the number of free aldehyde groups present and, hence, of the number of glucosidic bonds split, unless a technique for reducing groups is employed that is independent of the chain length of the oligo- or polysaccharide. This is true for the hypoiodite method of Willstätter and Schudel (*11*) and modifications thereof. The dinitrosalicylic acid method of Sumner (*12*), when maltose is used as a standard, has also been found to be practically independent of the chain length of the reducing saccharides, and hence to yield reducing values, which are fairly proportional to the number of reducing end groups (*13*), even at rather high molecular weights.

b. Decrease in Viscosity, or Liquefaction. Owing to the extremely rapid decrease of the giant particle size of a starch paste during α-amylatic attack, there is a rapid decrease in the viscosity during the initial stage of enzyme action, while the increase in reducing power is still hardly noticeable (*14*).

c. Change in the Iodine-Staining Properties. Owing to the rapid shortening of straight α-1,4-glucosidic chains of the substrate during α-amylatic degradation, there occurs a shift of the maximum of light absorption of the iodine-starch complex to lower wavelengths (*15*); i.e., a shift of the blue iodine color to purple and brown, followed by the complete disappearance of the capacity to produce a stain with iodine. The disappearance of the iodine color (achroic point) or its change can be measured either spectrophotometrically or by comparison with suitable artificial color standard solutions, and can thus be used for the quantitative determination of α-amylase action (*16–19*).

d. Decrease in the Turbidity of Glycogen Solutions. This decrease is due to the rapid decrease of the giant particle size upon α-amylatic action. It can be measured nephelometrically and thus serve as a means for activity determination of α-amylase.

e. Change in Optical Rotation. The reducing oligosaccharides are liberated in their optical α-configuration by the action of α-amylase, as it is to be expected from the α-1,4-glucosidic nature of these bonds in the polysaccharides; they undergo a downward mutarotation after their liberation.

2. *Nomenclature*

The liberation of the reducing saccharides in their α-configuration and the downward mutarotation of the reaction mixture are typical for α-amylatic action and contrast with the behavior of β-amylatic degradation. The latter is accompanied by a Walden inversion, thus releasing the reducing sugar in the β-configuration. On the basis of these phenomena, Kuhn (*20*) termed the two enzymes α- and β-amylases, respectively.

The reduction of viscosity, the change of iodine-staining properties, and the decrease in glycogen turbidity are consequences of the rapid transformation of high molecular weight substrate into lower molecular weight dextrins by α-amylase. This capacity of amylases is generally called dextrinogenic activity, in contrast to their ability to form reducing sugars, called saccharogenic activity. Accordingly, Ohlsson (*21, 22*) called α-amylase *dextrinogenic amylase* and β-amylase *saccharogenic amylase.*

3. *Mechanism of α-Amylase Action*

The action patterns of amylases are far more complicated than those of most other enzymes, especially those that act upon well-defined, homogeneous, and crystallizable substrates of low molecular weight and that form reaction products with like properties.

a. The Substrate. In contrast to the latter enzymes, amylases do not act upon a single substrate but rather on a variety of substrates; i.e., linear amylose, branched amylopectin, or mixtures of these two; highly branched glycogen; highly viscous starch pastes; native starch granules; or soluble starch with varying degrees of degradation, each substrate having its own mechanism of amylase action. The mechanism of amylase action is complicated because of the complexity of the chemical and physical nature of their substrates with respect to degree of branching and degree of polymerization, and in particular because of the *heterogeneity* of their substrates with regard to each of these two factors. Further confusion arises from the fact that some investigators consider amylose to be a product obtained by a particular process of extraction, rather than a chemical entity. In the view of these workers amylose may, therefore, contain certain quantities of the branched constituent of starch. It is more common and also more logical, however, to consider the term amylose a synonym of the linear α-1,4-glucosidic polysaccharide of starch, i.e., to apply the name of amylose to a chemical substance. Additional complications in the understanding of amylase action arise from the fact that amylose has the tendency to aggregate during standing into larger particles, i.e., to retrogradate during aging and thus to escape enzymatic attack. This phenomenon is not visible until precipitation occurs and has often been confused with a resistance of amylose toward enzymatic action because of a supposed presence of structural obstacles.

b. Initial and Final Stages of Amylase Action. There occurs a considerable change in the substrate of amylases during the early enzymatic reaction. A single amylatic attack on one molecule of substrate will yield two molecules of split products, which will most likely be different from one another, both of which may again be a substrate for further amylatic action, and so on until the reaction comes to an end. This succession of reactions can be represented by the following simplified expression: initial substrate → intermediate substrates → end products.

During the initial stage of the reaction, amylase will act upon substrates that are different from those existing during the final stages, and the mechanism of action may be different, therefore, during these two extreme stages. Thus, amylases of different sources which may behave in an identical fashion during the initial stage, may function differently during the final stages of the enzymatic degradation; i.e., they may yield different end products, a phenomenon frequently encountered and which is of great importance for our comparative considerations.

An explanation for this phenomenon has been given earlier (*8*). The action patterns of an amylase are most typical during the initial stage of the reaction during which the enzyme acts upon the original substrate or on products closely related to it. During this stage the affinity between enzyme and substrate will not influence the *course* of the reaction, i.e., the selection of the bonds to be hydrolyzed, but will govern only the *kinetics* of the reaction. Assuming that the affinity decreases continuously during the enzymatic reaction as the molecular weight of the intermediate substrates becomes smaller (*23–25*), a point will eventually be reached at which the affinity becomes so low that the enzymatic action will cease. For amylases with different substrate affinities, this end point will occur at different stages of the reaction, even though the initial action patterns of these enzymes were the same. It follows that the affinity between amylase and substrate will determine, at least to some extent, at what size of the split products the reaction will be arrested, i.e., what the end products will be.

c. α-Amylatic Degradation of Amylose. During the initial stage of the reaction a rapid random hydrolysis of α-1,4-glucosidic bonds occurs (*26*), whereby however the terminal bonds of the original and intermediate linear substrates are resistant to enzymatic attack (*27*). During this phase, the iodine-staining capacity of amylose disappears. The number of glucosidic linkages broken to reach this stage of the hydrolysis differs markedly for amylases from various origins (*28*), obviously because of their different affinities for the split products. In addition, the molecular weight of the substrate rapidly decreases until the polysaccharide has been broken down to maltose, maltotriose, and higher maltodextrins consisting mainly of maltotetraose (*27, 29–31*). The reaction then slows down considerably, but continues until only maltose (60%) and maltotriose (40%) subsist (*27*, *32*). During this phase, maltotetraose yields mainly maltose (*33*), maltopentaose is split into maltose and maltotriose, while maltohexaose is broken down to two molecules of maltotriose or, by an alternate mechanism, to maltose and maltotetraose (*34*), the latter being further hydrolyzed to maltose. Finally, in a very slow reaction, maltotriose is split to maltose and glucose. Thus, the ultimate products of α-amylatic degradation of amylose are 87% maltose and 13% glucose (*8*, *27*, *33–36*). Most of these observations were made with human salivary, hog pancreatic, or malt α-amylases.

Some discrepancies between the products obtained during the final stages of enzyme degradation have appeared in the literature. These are most likely due either to the presence of branched impurities in the amylose used for the experiments or to the failure to interpret properly or to exclude retrogradation of amylose. This latter phenomenon manifests itself by an incomplete enzymatic degradation of amylose and can be completely eliminated by one or several rejuvenations consisting in alkalinization of the digest, followed by neutralization and addition of enzyme (*37*).

d. α-Amylatic Degradation of Amylopectin and Glycogen. Only the α-1,4-glucosidic linkages of these substrates are hydrolyzed by the amylases, and all the branching points (α-1,6-glucosidic bonds) appear intact in the end products. As in the case of the α-amylatic degradation of amylose, amylopectin is broken down into dextrin in a rapid primary reaction whereby the α-1,4-glucosidic bonds are randomly hydrolyzed.

The terminal bonds of amylose are resistant to α-amylatic attack, and this is also true for the degradation of amylopectin and glycogen. In addition, the three α-1,4 links adjacent to the branching links behave like terminal links and are, in virtue of their position, equally resistant to hydrolysis by α-amylase (*38*). During the rapid primary reaction of the α-amylatic degradation of branched polysaccharides, the absorption maximum of the iodine complex shifts to lower wavelengths (*15*) while the intensity of the light absorption at the maximum decreases and finally disappears; i.e., the iodine color of amylopectin changes from purple over red to brown and then disappears, while that of glycogen just fades out. Simultaneously with the changes occurring in the iodine complexes, the molecular weight of the substrates rapidly decreases. The average chain length at which the iodine color of amylopectin turns to red (erythro point) or just disappears (achroic point) depends on various factors, such as the heterogeneity of the original substrate with regard to molecular weight, its degree of branching, to a lesser degree its tendency to retrogradation (*39*), and, finally, the presence of unbranched impurities. The latter have a higher tendency to retrograde than has amylopectin and, therefore, escape degradation; they then cause the iodine-staining property of the mixture to disappear more slowly than that of pure amylopectin during α-amylatic breakdown. Compared to the α-amylatic degradation of amylose, amylopectin is more slowly attacked by this enzyme (*39*), and the reaction slows down when 50 to 55% of the glucosidic bonds are split. The end products of exhaustive degradation by swine α-amylase of corn amylopectin with 4% branching points are 19% glucose, 73% maltose, and 8% isomaltose, whereas malt α-amylase leaves somewhat less glucose (13.5%) and larger amounts of α-1,6-glucosidic bonds containing dextrins (16.2%), having an average degree of polymerization of 2.8 (*39*). Potato amylopectin containing 0.4% glucose-6-phosphate (*40*) yields 13% glucose, 68% maltose, and 19% of branched dextrins with an average chain length of 6.2. It is obvious that the end products of α-amylatic degradation of glycogen are richer in branched dextrins and contain less glucose and maltose than the products of amylopectin degradation. Whelan and Roberts (*38*) found after exhaustive breakdown of liver glycogen by salivary amylase the following split products: maltose (4.57×10^{-3} moles); maltotriose (2.25×10^{-3} moles); and dextrins containing α-1,6-branch linkages, but no tetrasaccharide. The dextrins were characterized as consisting of a mixture of a total of eight different penta-, hexa-, hepta-, and octasaccharides, each containing one α-1,6-glucosidic link.

e. α-Amylatic Degradation of Starch Pastes. Starch pastes and soluble starch are essentially mixtures of amylose and amylopectin. Whereas a solution of soluble starch behaves as such, the enzymatic degradation of starch pastes represents a special case owing to the extremely high viscosity of this material and to its peculiar colloidal structure. The phenomena occurring during swelling of starch granules, and the existence in a starch paste of giant particles containing huge numbers of amylopectin and amylose molecules held together by crystalline regions, has been discussed by K. H. Meyer (*41*, *42*). The action of α-amylase on these giant particles produces a dramatic decrease of the viscosity, since the rupture of only minute numbers of bonds therein drastically reduces the particle size while the number of reducing end groups is not yet measurably increased.

Once these large particles have been broken down to the size of molecules, and the texture of the starch granule has disappeared, the remainder of the starch paste behaves toward the enzyme like soluble starch, i.e., like a mixture of amylopectin and amylose, and is further hydrolyzed accordingly.

4. *Distribution of α-Amylase*

This enzyme has been found abundantly throughout both the animal and the vegetable kingdoms.

a. Animal α-Amylases. In vertebrates α-amylase occurs in a great number of different tissues, organs, and fluids, but most of all in saliva and pancreas. Many times lower concentrations of amylase have been reported in the secretions of the small intestine, in liver, testes, thyroid, the ruminant stomach, and adrenals, and also in blood, urine, milk, cerebrospinal fluid, and in the chicken egg (*43*). Many of the older amylase determinations have been carried out in phosphate buffer, and may include, therefore, phosphorylase activity. Newer measurements (*44*) in Veronal buffer are more specific for amylase and yield results shown in Table II.

TABLE II

COMPARATIVE AMYLASE ACTIVITIES OF TISSUES, ORGANS, AND FLUIDS[a]

Source of amylase	Amylase activities in Somogyi units per 100 mg. tissue or 100 ml. fluid[b]					
	Rats			Mice		
	pH 7.0 Phosphate	pH 7.6 Veronal	Ratio	pH 7.0 Phosphate	pH 7.6 Veronal	Ratio
Serum	2,010	1,980	0.99	3,750	3,360	0.90
Lungs	663	641	0.97	1,690	1,590	0.94
Spleen	543	562	1.03	1,240	1,210	0.98
Heart	464	439	0.94	735	710	0.97
Kidney	652	638	0.98	890	840	0.94
Brain	52	56	1.08	95	91	0.96
Duodenum	66,400	65,700	0.99	12,240	12,500	1.02
Perirenal fat	644	628	0.98	—	—	—
Colon	3,960	3,880	0.98	1,340	1,310	0.98
Muscle	202	134	0.66	1,040	900	0.87
Liver	781	737	0.94	1,660	1,620	0.98
Pancreas	2,810,000	2,474,000	0.88	2,099,000	1,514,000	0.72
Saliva	626,000[c]	—	—	—	—	—

[a] According to McGeachin *et al.* (*44*).

[b] Determined according to the method of Van Loon *et al.* (*46*).

[c] Average of determinations in 15 individual animals, ranging from 91,000 to 1,340,000 units (*45*).

(*1*). *Salivary amylase.* The concentration of this enzyme, formerly also called *ptyalin*, depends very much on the animal species. According to Oppenheimer (*47*) three main classes of the potency of salivary amylase in various animals can be distinguished: (*a*) High activities in man and ape (*48*), in most fungivores and herbivores; i.e., lamb, rabbit, rat, mouse, guinea pig, squirrel (*49*); exceptions are the horse, beef, goat, rabbit, deer, and sheep (*50–52*). (*b*) Low activities in omnivores, i.e., swine, bear; in ruminants, i.e., beef, sheep, goat, deer. (*c*) No activity in carnivores; i.e., dog and cat (*53, 54*).

The activity of salivary amylase in the horse has been reported to be low by some investigators and inexistent by others (*55, 56*), but a zymogen has been described in the saliva of horses which is believed to be activated to amylase by oxygen (*43*).

Amylase has further been found in the saliva of amphibians, like the frog and toad (*57*), and in the oral mucosa of fishes (*43*), but no salivary amylase could be detected in birds (chicken) (*58*).

With certain exceptions some relationship appears to exist between the starch content of the food of an animal species and the activity of salivary amylase of the animal. It has to be realized, however, that the above classification is rough and does not apply to every animal species. Furthermore, considerable differences in the amylase potency exist within each group. Thus, it has been reported that the average salivary amylase activity in the rat (91,000–1,340,000/100 ml.) is several times that in human saliva (50,000–200,000 units/100 ml.) (*45*), but wide variations exist in both.

TABLE III
AMYLASE LEVELS OF MOUSE SUBMAXILLARY-SUBLINGUAL GLANDS (*59*)[a]

Strain	Number of animals	Mean amylase level[b]
CFW	22	0.15 (0.012)[c]
C57BL/6	14	0.14 (0.006)
C3H	35	0.19 (0.008)
DBA/1	41	0.10 (0.003)
F_1(C3H-DBA/1)	15	0.15 (0.008)

[a] Reproduced by courtesy of the Society for Experimental Biology and Medicine.

[b] Measured according to the method of Myers *et al.* (*60*) and expressed in terms of milligrams of reducing sugar found per milligram wet weight of gland.

[c] Standard error of mean in parentheses.

In the mouse, the submaxillary gland amylase levels were found to be genetically determined in four different inbred strains and in one hybrid strain, as seen from the data in Table III. These data were found to be consistent with a multiple and additive gene control;

histological examinations of glands from mice of each strain ruled out genetic differences in amount of connective tissue or number of ducts and acini (*59*).

Unlike pancreatic amylase, salivary amylase is present in the newborn and has even been found in the parotic gland of bovine embryos (*61*).

Salivary amylase in man originates mostly from the parotic gland (*62*), and both submaxillary and sublingual glands also contribute to the amylase secretion. Man produces about 1 liter of saliva from all three glands during 24 hours, containing approximately 0.4 gm. of pure amylase (*63*), together with about 3 gm. of other proteic material, in particular mucoids and mucoproteins, a few other enzymes such as certain proteases, an oxidase, lysozyme (*64*), and possibly hyaluronidase (*65*), and in addition, a number of inorganic salts, notably sodium chloride and potassium, calcium and magnesium phosphates, sulfate, bicarbonate, and thiocyanate. Human salivary amylase has been obtained in pure and crystalline form (*63, 66*).

(*2*). *Pancreatic amylase.* The richest source of animal α-amylases is no doubt the pancreas. In contrast to other pancreatic enzymes, in particular proteases, amylase does not occur in form of a zymogen in the pancreatic secretion but has always been observed therein as the active enzyme.

Pancreatic amylase has been found in the order of decreasing activity in swine, dog, beef, and sheep (*67*), in man, the horse, and further in the rat, mouse, chicken, dog, and guinea pig (*44*) (see also Table IV). The pancreatic enzyme has been reported to occur early in bovine embryos (*61*), as does salivary amylase, whereas other workers claimed that the pancreatic secretions of the newborn do not contain amylase, which arrives therein only after several months (*43*).

It should be noted that although amylase generally occurs in the pancreas of those animal species where it also exists in the saliva, it is found in addition in animals where no salivary amylase can be detected, i.e., in the dog, horse, beef, etc.

The synthesis and secretion of amylase by pigeon pancreatic slices has been studied *in vitro* by Hokin (*68*). An interesting relationship between pancreatic amylase and thyroid function was described by Townsend *et al.* (*69*), who found that thyroidectomy, with or without I^{131}, reduces both the pancreatic weight and the amylolytic activity.

Pancreatic amylase from various species has been obtained in pure and crystalline form; i.e., from swine (*70–72*), man (*73, 74*), and rat (*75*). Although the proteic nature of pancreatic amylase has been well established (*71, 76, 77*) and the amino acid composition of crystalline

TABLE IV

AMYLASE CONTENT[a] OF TISSUES AND ORGANS IN VARIOUS ANIMALS[b]

Animal	Serum	Lung	Spleen	Heart	Kidney	Muscle	Liver	Brain	Duodenum	Perirenal fat	Colon	Pancreas
Rat	2730	760	570	500	870	190	1820	82	63600	1310	3500	2,750,000
Mouse	3640	1790	830	800	900	1140	1910	100	15000	—	1990	2,110,000
Guinea pig	2210	910	320	310	880	970	1340	81	3750	182	550	280,000
Dog	490	250	108	121	204	102	227	33	2190	114	356	496,000
Chicken	830	320	131	198	271	36	383	35	2300	—	—	1,420,000

[a] Expressed in Somogyi units per 100 mg. tissue or 100 ml. fluid.

[b] According to McGeachin *et al.* (*44*).

preparations of this enzyme has been determined (see Table V), earlier reports seemed to indicate that this enzyme is not a protein (*78*).

This observation was probably caused by the fact that the authors had determined the protein content of their preparation on dialyzed samples after the activity measurements had been carried out on undialyzed aliquot samples. During the dialysis, the amylase has supposedly been broken down by proteolytic enzymes, which have been found to accompany even certain crystallized preparations of swine pancreatic amylase (*71*). Metal binding agents have been reported to enhance the susceptibility of the amylase for proteolytic attack, whereas bivalent metals have been found to abolish it, thus stabilizing amylase.

TABLE V

Amino Acid Composition of Crystalline Amylase from Swine (*77*)[a]

Amino acid	Grams per 100 gm. protein	Moles per 10^5 gm. protein
Cystine	2.3	9.58
Methionine	2.1	14.1
Histidine	3.9	25.1
Tyrosine	5.3	29.3
Proline	3.6	31.3
Threonine	3.9	32.7
Tryptophan	6.7	32.8
Arginine	5.8	33.3
Lysine	4.9	33.5
Serine	4.1	39.0
Phenylalanine	10.1	61.2
Valine	7.8	66.6
Glutamic acid	10.5	71.4
Alanine	6.9	77.4
Leucines	11.5	87.7
Glycine	6.7	89.2
Asparagine	12.4	94.1
Free aspartic acid	2.0	14.8

[a] Reproduced by courtesy of the American Chemical Society.

Inositol had been reported to be an esential component of pancreatic amylase (*79, 80*), but pure and crystalline preparations of this enzyme proved to be free of inositol (*71, 81*).

The primary amino groups of swine pancreatic amylase have been found to be essential to the enzymatic action, but the hydroxyl groups of the tyrosine residues do not appear to affect its activity (*82*).

(3). *Blood amylase.* In blood, amylase has been reported to occur in the plasma, in erythrocytes, and in leukocytes. Wagner has shown (*83*) that the enzyme in human white blood cells breaking down glycogen is actually a phosphorylase, rather than an amylase as was originally postulated by Willstätter and others (*84, 85*).

In the cytoplasm of nucleated and anuclear erythrocytes of vertebrates small amounts of an enzyme have been described which liberate glucose from glycogen (*86*). This enzyme is believed by the author to be an amylase, presumably an α-amylase, and has been found in man, mice, chicken, toads, newts, and certain freshwater fishes (see Table VI).

TABLE VI

AMYLASE CONTENT IN THE CYTOPLASM OF ERYTHROCYTES (*86*)

Red cells	Amylase activity[a]
Man	4.9
White mouse	4.5
Chicken	5.8
Lacerta	8.6
Toad	9.2
Newt	9.6
Carassius auratus	16.6

[a] In γ-glucose split off from 1 mg. of glycogen by 1 mg. protein nitrogen in 1 hour at 40° at pH 7.2.

Plasma or serum amylase has been studied predominantly in man, in view of possible diagnostic and other clinical implications of the deviation in its level from the normal state (*87*). Thus, Short (*88*) reported that high serum amylase developing within the first 2 hours of the onset of an acute abdominal condition, in the absence of renal failure or morphine sedation, is diagnostic of pancreatitis. Transient and moderate to large changes in serum amylase levels were observed after cholecystotomy with return to normal within 10 days (*89*). In diabetic patients not treated with insulin, serum amylase was reported to be higher than normal, though amylase levels were normal in insulin-treated patients (*90*). Studying a large patient population, Somogyi (*91*) has found, however, that blood amylase is lowered in the majority of diabetic patients. The amount of plasma amylase in normal individuals has been estimated to be over 0.0001% of the total plasma protein (*92*).

Serum amylase in animals has been reported in the rat, mouse, guinea pig, dog, and chicken (see Table IV) (*44*), and also in the calf, cow, and sheep, etc. (*93*). Unlike salivary amylase, serum amylase has not been reported to be absent in certain animal species, and this enzyme, like pancreatic amylase, must be considered to occur in all vertebrates. That amylase does not enter the blood stream through the pancreatic duct has been concluded from experiments in which the concentration of blood amylase of dogs, although eight to sixteen times increased a few days after complete ligation of the pancreatic duct, returned to normal between the sixth and eighth day (*94*).

(*4*). *Urinary amylase.* Urinary amylase is generally found in all animal species having pancreatic amylase, and it is usually assumed that the level of urinary amylase parallels the amylase activity of the pancreas. Roe *et al.* (*95*) observed, however, that pancreatectomy of rats is not followed by a decrease in the urinary enzyme and concluded that the pancreas is not an important source for urinary amylase in the normal rat. This is in contrast to older reports according to which urinary amylase is decreased after extirpation of the pancreas (*96*), but is increased after ligation of the pancreatic ducts (*97*).

(*5*). *Liver amylase.* Liver amylase has been reported in many animals (*98*), in particular in the dog and, in order of decreasing activity, in swine and in rabbit and lamb (*99*); it has been identified as being an α-amylase (*100*), but the last doubt about this enzyme being a phosphorylase has been removed by the work of McGeachin *et al.* (*44*), who determined its activity in the absence of phosphate (see Table II).

(*6*). *Intestinal amylase.* Intestinal amylase was identified in histological sections of mucosa of the duodenum and ileum of the rat (*101*). The amylase content of the Thiry-Vella juice of the dog was found to increase with the starch content of the food, whereas the amylatic action of the juice was the lower the farther away the fistula was from the duodenum (*102*). Duodenal amylase levels were reported to be very high in the rat, high in the mouse, and much lower in the guinea pig, chicken, and dog (see Table IV). No amylase has been detected in the duodenal juice of the sheep (*103*).

(*7*). *Animal amylases from miscellaneous other sources.* Many other organs have been reported to contain α-amylase. Frequently, it is not clear whether the starch or glycogen degrading activity should be ascribed to a hydrolytic or a phosphorolytic enzyme. Repeated reports have appeared in the literature stating that muscle contains amylase besides phosphorylase (*104, 105*), although the amounts of muscle amylase in the rat and mouse are very low (see Table II) (*44*). Amylase has been observed to occur in the kidney, heart, thyroid, and testes (*106*). The amounts of amylase in the spleen, heart, kidneys, muscle, brain, perirenal fat, and colon of the rat, mouse, guinea pig, dog, and chicken have been tabulated by McGeachin *et al.* (*44*) (see Table IV) and are very low compared to the amounts of pancreatic amylase.

It is not exactly known whether these minute amounts of α-amylase actually originate from the tissues or are released from microorganisms growing there. Amylase-producing bacteria have, indeed, been observed in the rumen of the sheep (*107*) and the cecum of the pig (*108*). Considerable amylase activity was observed in the bile of sheep, whereas bovine bile contained only traces (*103*).

Honey has been found to contain amylase, and it has been demonstrated that this enzyme therein actually originates from the bee (*109*).

Amylase has been found in mollusks: it could be extracted from styles of *Meretrix luzoria* and *Mactra veneriformis* (*110*).

In protozoa, amylase has been detected and localized in amebas (*111*).

The role of amylase during embryonic development has been investigated repeatedly. Amylase activity has been found to be very high in the egg of the toad (*Bufo vulgaris*) during cleavage, and to decrease progressively until the end of embryonic life. This is followed by a rapid increase in amylase at the beginning of larval life (*112, 113*).

b. α-Amylases in Higher Plants. The most important occurrence of α-amylase in higher plants is in germinated cereals, where it is associated with considerable amounts of β-amylase. Whereas the latter enzyme is present also in the ungerminated cereals, α-amylase activity is practically absent at that stage, and the low amounts of α-amylase that can be detected before germination are believed to be derived from the microflora on the grains (*114*). Schwimmer (*115*), without regard to the origin of the small amounts of α-amylase present in wheat kernels, observed that α-amylase per kernel remains relatively constant during the entire course of development and the early stages of ripening, whereas the amylase content per dry weight decreases during this period, because of the considerable weight changes of the kernels (see Table VII). At the very early stages, the amount of α-amylase per dry matter

TABLE VII

α-Amylase in Developing Wheat of Baart I (*115*)[a]

Time after harvest (days after blossoming)	α-Amylase content × 10^4	
	Per 1000 kernels	Per grain dry weight
24	27	13.5
41	26	5.2
85	32	1.0
116	28	0.7
168	10	0.2

[a] Reproduced by courtesy of *Cereal Chemistry*.

is about 5–10% of that found in germinated wheat and decreases to one-seventieth of that value at complete maturity. The presence of α-amylase in the early stages of ripening of wheat and its disappearance during further ripening has also been observed by other workers (*116*).

Ungerminated corn, millet, sorghum, and rice have been reported to be practically devoid of both α- and β-amylases (*117*). It is during the process of germination that the majority of α-amylase activity develops in cereals. The reason for this phenomenon has been abundantly discussed though no conclusive evidence for the mechanism underlying the appearance of α-amylase activity during germination is available. One hypothesis is that a substantial part of α-amylase of the grain is inactive and insoluble in water. This "latent" enzyme is bound in some way to insoluble protein and can be rendered soluble and active by the action of proteolytic enzymes such as papain (*118*). According to other sources, both α- and β-amylases are said to exist in the early stages of

TABLE VIII

EFFECT OF GERMINATION ON FREE AND TOTAL α- AND β-AMYLASES OF WHEAT[a]

Germination moisture (%)	Time (Days)	α-Amylase Free	α-Amylase Total	α-Amylase Free as % total	β-Amylase Free	β-Amylase Total	β-Amylase Free as % total
	0	—	—	—	3.8	20.0	19
35	2	3.8	4.6	83	4.5	19.6	23
	3	9.3	10.5	88	4.9	19.7	25
	4	17.9	21.2	84	5.4	20.3	27
	5	28.8	30.8	93	6.6	20.4	32
	6	43.8	42.0	104	7.6	20.6	37
40	2	13.6	15.3	89	4.6	20.1	23
	3	36.6	36.7	100	6.0	20.6	29
	4	58.6	58.6	100	7.8	20.5	38
	5	68.5	71.9	95	9.1	20.5	44
	6	89.6	91.0	98	10.1	20.5	49
45	2	19.0	19.0	100	6.7	19.8	34
	3	39.8	39.6	100	8.3	20.2	41
	4	61.4	65.2	94	9.5	20.4	47
	5	76.1	75.3	101	10.2	20.4	50
	6	91.4	96.1	95	10.3	21.0	49

[a] According to Geddes (*119*). Reproduced by courtesy of Interscience Publishers, New York.

wheat, but the former enzyme disappears again during further ripening (*116*). Careful studies by Geddes *et al.* (*119*) seem to show, however, that there is an actual synthesis of α-amylase during the germination period of wheat. Their results shown in Table VIII appear to confirm the general trends noted by Kneen *et al.* (*120*).

Similar conditions of the appearance of α-amylase have been observed during germination of other cereals. Thus α-amylase is known to occur in the germinated forms of barley (barley malt) (*21*, *22*, *121*, *122*), wheat (*120*, *123*), rye (*124*), oat (*125*), rice (*126*), maize (corn), sorghum, and millet (*127*). The α-amylase from barley malt has been obtained in the pure and crystalline state (*128–130*).

It was found that wheat and rye damaged by sprouting contain ten to a hundred times more α-amylase than the undamaged grain (*131*).

Amylatic activity mostly α-amylase, has been described in maple and birch saps (*132*, *133*), and also in the tobacco leaf. The maximum activity in the latter was found in the ripest leaves; leaves with tobacco mosaic virus have higher amylase activity than healthy ones (*134*).

Seeds of *Canavalia ensiformis* were reported to contain α-amylase. This study was carried out on seeds from East Africa that could no longer germinate. The enzyme was found only in the cotyledons, not in the embryo (*135*).

c. Bacterial α-Amylases. The study of starch degradation by bacteria goes back as far as 1819 (*136*).

A large number of bacteria are known to release starch-degrading enzymes into the culture medium. Among more than 1000 bacterial isolates investigated by Peltier and Beckord (*137*), 37 were found to possess high dextrinizing power and, hence, to contain α-amylase, and 168 more were able to hydrolyze starch. Kneen and Beckord (*138*) classified these bacterial amylases into four groups. One is called the saccharifying type of *Bacillus subtilis* amylase, which is or contains β-amylase and which will be discussed together with other β-amylases. Another type of Kneen and Beckord's classification is the "amylase" of *B. macerans* which, as was pointed out earlier, is not a hydrolase and will not be included in the present chapter. This enzyme actually belongs to the class of transferases, although its unfortunate name always produces confusion with hydrolytic enzymes. The transferase nature of *B. macerans* "amylase" has been convincingly demonstrated by French *et al.* (*139*), and the name cyclodextrinase has been suggested for this enzyme (*8*). The two other classes of bacterial amylases, the actual bacterial α-amylases, are of the *B. subtilis* and of the *B. polymyxa* type.

(*1*). *B. subtilis amylase.* This enzyme is noted for its high-temperature stability (*140*, *141*) and is known for being active even at 80° C.

This property cannot be regarded, however, as a characteristic of the active protein itself since crystallized amylase from *B. subtilis* is much less resistant to temperature and loses its activity at 35° (*8*). It is more likely that the crude enzyme contains a stabilizer that is removed during purification.

The activity of this enzyme, which was found to be an α-amylase (*140, 141*), depends on a number of functional groups in the protein. Thus, —SH and primary $—NH_2$ appear to be essential for enzyme activity (*142*), and tyrosyl hydroxyl has been reported to be also necessary (*143*), whereas some ε-amino groups of lysine only increase the stability of the enzyme. The influence of the culture medium on the production of amylase (*144*) has been studied in detail. Commercial preparations of *B. subtilis* amylase are known under the names Rapidase, Superclastase, Biolase, and Liquefase (*145*). α-Amylases from Biolase and from a bacterial amylase concentrate from Takamine Laboratory have been obtained in the crystalline state (*146, 147*). *B. mesentericus* amylase is very similar to the enzyme from the culture medium of *B. subtilis* (*148, 149*).

(*2*). *B. polymyxa Amylase. B. polymyxa* amylase has been reported to be much less heat resistant than the *B. subtilis* enzyme (*150*). It is for this reason that Kneen and Beckord classified this enzyme as a separate group. Since the heat resistance of *B. subtilis* amylase does not appear to be a property of the enzyme protein itself, but rather an environmental protective effect, this differentiation between *B. subtilis* and *B. polymyxa* amylases should not be interpreted as an evidence of differences in their nature, activity, and mechanism of action, in particular since the behavior of these two amylases is otherwise very similar. *B. polymxa* amylase develops in a culture medium containing peptone and starch (*151*).

(*3*). *Amylase from Clostridium butyricum.* Amylase from *Clostridium butyricum* has been isolated by Hobson and MacPherson (*152*) and has been found to be free of β-amylase and maltase. The occurrence of this microorganism in the rumen of the sheep (*107*) and in the cecum of the pig (*108*) may well account for the existence of α-amylase in these sites.

(*4*). *Amylase from Clostridium acetobutylicum.* Amylase from *Clostridium acetobutylicum* has been found to be associated with considerable amounts of maltase and an α-1,6-glucosidase that hydrolyzes the branching points of starch (*153*). Consequently, the end products of enzymatic action of this mixture are mostly maltose (*154*).

(*5*). *Miscellaneous bacterial α-amylases.* Many other bacteria have been found to emit α-amylase into the culture medium, e.g., *Bacillus* *amyloliquefaciens*, a variety of *B. subtilis*, secretes a small amount of amylase when shaken aerobically in the presence of phosphate (*155*). The effects of carbon sources and base analogs of nucleic acid on the amylase formation have been studied and have been found to be of great importance (*156*). *Pseudomonas saccharophila* also emits an α-amylase into the culture medium (*157*).

d. Mold α-Amylases. A great number of molds are known to produce α-amylase. *Rhizopus japonicus* contains α-amylase, but there is probably also some β-amylase present (*158*). The best known of all amylases of mold occurs in *Aspergillus oryzae*. This microorganism has been known in Japan for more than 2000 years under the name *koji* (*159*) and has been used in the manufacture of alcoholic beverages called sake. Details on the preparation of *koji* and a full description of its amylase have been given by Atkinson (*160, 161*). Later, Takamine (*162*) investigated this enzyme further, compared its action to that of malt amylases, and finally brought on the market a commercial preparation of the enzyme, called Taka-amylase, for use in the textile industry (*163*). The shortage of barley malt in the United States during World War II led to extensive research through which the commercial production of mold bran on a large scale became possible (*164*). This mold bran was used in the manufacture of industrial alcohol from grains (*165*).

The purification and crystallization of α-amylase from *A. oryzae* has been achieved in several laboratories (*166–169*), and the properties and action patterns of the pure (*36, 170, 171*), as well as of crude preparations have been described (*172–176*).

Certain strains of *A. niger* have also been reported to produce starch-hydrolyzing enzymes in substantial amounts (*177*).

The amylolytic system in the black-*koji* molds of the *A. oryzae* and of similar species such as *A. awamori*, *A. usamii*, etc., has been extensively studied by the Japanese workers (*178–180*).

e. Yeast Amylases. In a thorough investigation of 64 different yeast species, Smits Van Waesberghe (*181*) came to the conclusion that the starch-degrading yeast enzymes are phosphorylases rather than amylases. These are supplemented by various glucosidases.

The enhancement of amylase action by yeasts has been known for a long time and has been explained by Schwimmer (*182*) and by Pan *et al.* (*183*) on the basis of the removal by yeast of maltose formed during amylatic digestion. That maltose inhibits the action of amylase is well known (*25*).

A report of the presence of extracellular amylase in *Endomycopsis fibuliger* (*184*) should be considered with caution. The action observed may well be due to phosphorylase.

5. *Properties of and Comparison between Various α-Amylases*

a. Mechanism of Enzyme Action. Because the nature of the substrate changes continuously during the action of α-amylase, the enzyme-substrate affinity varies considerably and progressively throughout the whole α-amylatic breakdown of starch or glycogen (8), and an adequate comparison of the mechanism of action of this enzyme from different origins can be made only when the different stages of the enzymatic reaction are considered separately. For this reason, an analysis will be made for (1) the initial stage of enzyme action involving the original undegraded substrate; (2) the intermediate stages approximately where the molecular weight of the substrate has been reduced considerably to produce dextrins that have just lost their property to stain with iodine (achroic point), but which can still be further broken down by the enzyme; and (3) the final stage of the enzymatic action at which the rate of reaction has practically come to a standstill, leaving behind the end product of the degradation.

(1). The initial stage of enzyme action. This phase is best characterized by the ratio of saccharifying to dextrinizing activity. The former represents the increase in reducing power, the latter the change in the iodine-staining properties of the substrate (*185*); both activities are measured while less than 15% of the glucosidic bonds of the substrate are hydrolyzed, with the use of "soluble starch" as the substrate, i.e., a chemically degraded mixture of amylose and amylopectin whose tendency to retrogradate is extremely low. The data in Table IX clearly show that the ratio of saccharifying to dextrinizing activity is the same for a large number of pure α-amylases originating from the most diversified sources. This leads to the conclusion that during the initial stage of starch degradation none of the α-amylases seem to favor or to neglect the hydrolysis of any particular bond to a higher degree than the other α-amylases from different origins; in other words, the initial mechanism of attack appears to be the same for all α-amylases, independently of their origin.

(2). The intermediate stages of α-amylase action. In a study on the action of several α-amylases upon amylose, Kung *et al.* (*28*) came to the conclusion that α-amylases from different sources differ markedly in their action. There is no doubt that these data, shown in column 3 of Table IX, are correct and that the phenomena observed are real. More than one interpretation of these data is possible, however. It appears to the writer that owing to the extremely high tendency of amylose to retrogradate, a portion of the substrate may escape enzymatic degradation for some time because of the formation of enzyme-resistant substrate and

TABLE IX

COMPARISON OF THE ACTION OF VARIOUS α-AMYLASES

Source of α-Amylase	Ratio of saccharifying to dextrinizing activity (*185*)	% Glucosidic linkages broken at the achroic point (*28*)	End products of exhaustive degradation of corn amylopectin (*36*, *39*)				Michaelis-Menten constant (gm./100 ml. × 10^2) (*186*)
			Glucose	Maltose	Dextrins		
			(%)	(%)	(%)	(D.P.)[b]	
Pig pancreas	9.75	23	18.5	73.2	8.3	2.2	1.8
Pig saliva	—	20.5	—	—	—	—	—
Human pancreas	9.7	14.5	—	—	—	—	—
Human saliva	9.5	14.5	—	—	—	—	—
Rat pancreas	—	15	—	—	—	—	—
Rat serum	—	15	—	—	—	—	—
Rat spleen	—	15	—	—	—	—	—
Rat liver	—	15[a]	—	—	—	—	—
Barley malt	9.7	—	13.5	70.3	16.2	2.8	7.8 (*187*)
B. subtilis	9.8	12	—	—	—	—	6.3
A. oryzae	9.8	12	9.5	63.7	26.5	5.5	3.5 (*36*)

[a] Extrapolated.

[b] Average degree of polymerization.

may thus delay the arrival of the achroic point. Depending largely on the over-all rate of enzyme hydrolysis, this phenomenon would be more important at high enzyme concentrations at which the amylose has little chance to retrogradate, whereas low enzyme concentrations would favor the formation of enzyme-resistant, retrogradated amylose (*188*). In the light of this explanation, the data of Kung *et al.* may not have much meaning.

If not caused by retrogradation, however, this phenomenon may be due to actual differences in the enzyme-substrate affinities for various α-amylases, causing differences in the products of the enzyme reaction, beginning at and during the intermediate stages of α-amylatic degradation.

(*3*). *The final stage of α-amylase action.* Although the differences between various α-amylases with regard to enzyme-substrate affinities may begin to manifest themselves during the intermediate stages of enzyme action, they will become even more important during the final stages, thus governing the nature of the end products of exhaustive enzymatic digestion. Some of the results on the end products with the use of pure enzymes are shown in Table IX. Obviously, α-amylase of *A. oryzae* yields three times as much dextrin having a 2.5 times higher degree of polymerization than pig pancreatic amylase, and malt amylase assumes an intermediate position. It would appear, therefore, that the pig pancreatic enzyme has a higher affinity for the substrate (in this case the low molecular weight dextrins) than malt amylase; this is in agreement with the higher Michaelis-Menten constant of the latter enzyme shown in the last column of Table IX.

It appears, therefore, that the mode of action of α-amylases from different sources on a given substrate is the same, but that the influence of their different enzyme-substrate affinities becomes more and more important as the enzymatic reaction proceeds and as the molecular weight of the substrate decreases. The enzyme-substrate affinities appear to vary with the origin of the α-amylases, thus causing differences in the end products and possibly also in the dextrins appearing during the intermediate stages.

b. Properties of the Enzyme

(*1*). *Enzymatic properties of α-amylase.* These are listed in Table X. Most of the data have been obtained with the pure crystalline enzymes. There appears to be a distinct difference between the animal α-amylases and those of plant or microbial origin; the latter have a lower ratio of specific activity per protein, a lower pH optimum of activity than the former, and do not require chloride for their activity.

(*2*). *Physicochemical properties.* As shown in Table XI, the solubility in water exhibits a similar differentiation between amylases from animal and vegetable kingdoms, and the dispersion constant, calculated from the optical rotation, is different in pancreatic and mold amylases, the two α-amylases in which it has been studied (*190*). The other characteristics appear to be more or less independent of the origin of the enzyme.

(3). *Stability of α-amylases.* This depends to a large extent on the degree of purification of the enzyme, as well as on the purification and crystallization procedures used. Crude *B. subtilis* amylase, for instance, is much more stable at 70° than the crystalline enzyme (*8, 189*).

TABLE X
ENZYMATIC PROPERTIES OF α-AMYLASES

Source of α-amylase	Specific activity per mg. protein	pH Optimum of activity	Activation energy (*170*) (cal./mole)	Turnover number	Activation by	
					Cl^-	Ca^{2+}
Pig pancreas (*71*)	630	6.9	13,500	25,000	+	−
Human pancreas (*74*)	980	6.9	13,350	—	+	−
Human saliva (*66*)	980	6.9	13,350	—	+	−
Human serum (*92*)	—	6.9	—	—	+	−
Barley malt (*129, 130*)	315	4.7–5.4	7,050	19,000	−	+
B. subtilis (*189*)	500	5.3–6.8	13,350	—	−	−
A. oryzae (*170*)	310	5.5–5.9	10,650	—	−	−

The influence of bivalent ions sequestrating agents, like ethylenediaminetetraacetic acid (EDTA) has been studied by Stein and Fischer (*193*). These authors observed that the resistance of crystalline amylases toward proteolytic enzymes is considerably decreased by the presence of EDTA (see Table XII). They assert that all α-amylases contain traces of Ca^{2+}. When Ca^{2+} is removed by metal-binding agents, a "metal-poor" amylase results which becomes exceedingly susceptible to proteolytic attack. Proteases are believed to accompany the crystalline enzymes in small and variable amounts as contaminants, thus accounting for the differences of stability in the presence of EDTA for amylases from various origins and for crystalline enzymes obtained by various procedures. The stabilization of amylases by calcium and by other bivalent metals, on the other hand, is regarded as an increase in resistance toward proteolytic attack.

c. Chemical Composition of α-Amylases. Table XIII indicates the composition of crystalline α-amylases with respect to nitrogen, phosphorus, calcium, and the requirement of sulfhydryl, amino, or tyrosyl hydroxyl groups for their activity. The amino acid composition of crystalline α-amylase from pig was shown in Table V.

d. Immunological Behavior. That crystalline hog pancreatic amylase

TABLE XI

PHYSICOCHEMICAL PROPERTIES OF α-AMYLASES

Source of α-amylase	Solubility in H_2O at 2°C. pH 7.0 (gm./100 ml.)	Electrophoretic mobility at pH 7.9 $\mu = 0.1$ (cm.^{2} volt^{-1} sec.$^{-1}$ $\times 10^{-5}$)	Iso-electric point	Diffusion constant $D_{20} \times 10^7$ (cm.^{2} sec.$^{-1}$)	Sedimentation constant, S	Molecular weight[c]	Dispersion constant, λ_0 (*190*)
Pig pancreas (*71*)	0.6	3.1	5.2–5.6	8.05 (*191*)	4.50 (*191*)	45,000 (*191*)	243
Human pancreas (*74*)	0.3	3.2	5.2–5.6	—	—	—	—
Human saliva (*66*)	0.3	3.2	5.2–5.6	—	—	—	—
Barley malt (*129, 130*)	15	3.1	5.7	6.53 (*192*)	4.52 (*192*)	59,500 (*129*)[b], 54,000 (*192*)	—
B. subtilis (*189*)	6	2.4–3.0[a]	—	—	5.4	—	—
A. oryzae (*170*)	10	4.2	4.2	—	—	—	271

[a] pH 8.55.

[b] From osmotic pressure measurements.

[c] From sedimentation data.

has antigenic properties, when injected into rabbits, has been demonstrated by McGeachin and Reynolds (*194*). These authors were able to inhibit a sample of hog pancreatic amylase to the extent of 95% by precipitation with the specific rabbit antiserum. They further observed that their antiamylase was able to inhibit to a considerable extent hog amylases from various other tissues or fluids, with the exception, however, of hog liver amylase (see Table XIV), and they concluded that hog amylases are more species specific than organ specific and that the liver enzyme appears to be different from the amylases of the other

TABLE XII

STABILITY OF α-AMYLASES

Source of α-amylase	pH Optimum of stability	Stability[a] in the presence of EDTA[b] (*193*)	Stabilization by
Pig pancreas (*71*)	7.0–8.5	0, 30[c]	Ca^{2+} [e]
Human pancreas (*74*)	4.8–11	—	—
Human saliva (*66*)	4.8–11	100	Ca^{2+}
Barley malt (*129, 130*)	4.9–9.1	—	—[f]
B. subtilis (*189*)	4.8–12[d]	82.5, 92[c]	Ca^{2+}
A. oryzae (*170*)	5.5–8.5	100	Ca^{2+}

[a] Approximate percentage of activity recovery after 1 hour at pH 7.2, 25°, in the presence of 0.005 *M* EDTA.

[b] Sodium ethylenediamine tetraacetate.

[c] The two figures represent two amylase preparations obtained by different methods.

[d] In the presence of Ca^{2+}.

[e] Also by Mn^{2+}, Ba^{2+}, Mg^{2+} and Ni^{2+}.

[f] Requires Ca^{2+} for activity.

TABLE XIII

CHEMICAL COMPOSITION OF α-AMYLASES

Source of α-amylase	% N	% P	Metal (*193*)[a]	Groups essential for activity		
				—SH	$—NH_2$ (primary)	—OH (tyrosyl)
Pig pancreas	15.8	0.05	Ca^{2+}	–	+ (*82*)	–
Human pancreas	15.8	0.01	Ca^{2+}	–		
Human saliva	15.8	0.01	Ca^{2+}	–		
Barley malt	13.4	0.01	Ca^{2+}	–		
B. subtilis	15.8	0.01	Ca^{2+}	+ (*142*)	+ (*142*)	+ (*143*)
A. oryzae	12.9	—	Ca^{2+}	–		

[a] At least 1 gm. atom firmly bound calcium per mole protein.

tissues and fluids. The same antiamylase was able partially to inhibit human serum amylase and serum amylases from the monkey and the dog, but not, or only to a negligible extent, the serum enzymes from the rabbit, rat, guinea pig, and mouse (see Table XV).

TABLE XIV
Percentage Inhibition of Amylases by Rabbit Antiserum to Crystalline Hog Pancreatic Amylase (*194*)[a]

Source of amylase	Hog		Dog		Rat	
	1:20[b]	1:100[b]	1:20[b]	1:100[b]	1:20[b]	1:100[b]
Pancreas	95	91	38	8	9	5
Saliva	95	—	—[c]	—[c]	5	2
Serum	93	75	35	13	4	3
Liver	4	1	13	8	3	5
Kidney	70	35	22	15	5	6
Spleen	93	70	66	39	24	15

[a] Reproduced by courtesy of the *Journal of Biological Chemistry.*
[b] Dilution of antiserum.
[c] Dogs have no salivary amylase.

An antiserum against crystalline α-amylase from *B. subtilis* has been prepared by Nomura and Wada (*195*). They found 90% inhibition of *B. subtilis* amylase by the rabbit antiserum, while the α-amylases from *A. oryzae*, *A. usamii*, and malt were not inhibited.

TABLE XV
Effect of Rabbit Antiserum, Specific to Crystalline Hog Pancreatic Amylase, on Mammalian Serum Amylases (*194*)[a]

Serum from	Percentage inhibition by antiserum 1:20
Hog	95
Rabbit	0
Dog	35
Rat	4
Human	25
Monkey	30
Cat	11
Guinea pig	5
Mouse	3

[a] Reproduced by courtesy of the *Journal of Biological Chemistry.*

6. *Conclusions on the α-Amylases*

This class of polysaccharidases is widely distributed through both the animal and vegetable kingdoms and occurs in many different animal tissues and fluids. Whereas human salivary and human pancreatic amylases have been found to be identical on the basis of chemical and physical tests (*74*), and the pancreatic, salivary, serum, kidney, and spleen α-amylases of the hog were reported to be equal, hog liver α-amylase is clearly different from all the other porcine amylases (*194*). Monkey and human serum amylases gave a similar and limited response to the antiserum obtained with hog pancreatic amylase, but rat, guinea pig, and mouse serum amylases were not inhibited by this antiserum, a result which was to be expected on the basis of the phylogenetic relationships.

The apparent identity in one and the same animal species of the α-amylases of all tissues and fluids, with the exception of liver amylase, points to the view that the low levels of α-amylase in serum, urine, lung, spleen, heart, kidney, brain, duodenum, colon, muscle, etc., represent mere "leakages" from the main sources; i.e., from pancreas and in some species from the salivary glands, whereas liver amylase being different from the others must be synthesized *in situ* and probably has some specific function in the physiology of the liver. A possible requirement of this organ for α-amylase might be the regulation of glycogen synthesis catalyzed by phosphorylase. As is well known, the latter enzyme requires carbohydrate primers to initiate the reaction. If these primer carbohydrates were depleted, the glycogen synthesis would either stop or, in the presence of stored glycogen, would merely further increase the molecular weight of the latter *ad infinitum*, while the *number* of glycogen molecules would remain constant. The presence of a small amount of α-amylase is sufficient, however, to produce new primer molecules and to protect against an increase of the molecular weight beyond certain limits.

Various enzymatic or physicochemical properties of the pure enzymes permit distinguishing between the animal α-amylases and those of the vegetable kingdom. The former have a high specific activity per milligram protein, an optimum of action at pH 6.9, a low solubility in water and require chloride for activity. On the other side, the plant and microbial α-amylases have lower specific activities per milligram protein, a pH optimum between 4.7 and 5.9, a ten- to twentyfold higher solubility in water and do not require chloride.

The mechanism of enzyme action appears to be the same for all α-amylases during the initial stages of the reaction. As the substrate is broken down into fragments of considerably smaller molecular weight, however, the difference in enzyme-substrate affinities between the α-amylases of various origins starts to become manifest and to cause differences in the end products remaining after exhaustive starch digestion.

B. β-AMYLASES

1. *Changes in the Properties of the Substrate Caused by the Action of the Enzyme*

The patterns of the changes in reducing power, viscosity, iodine staining, and optical rotation of the substrate caused by β-amylatic degradation are significantly different from those observed during the action of α-amylase.

a. Increase in Reducing Power. As in the case of α-amylase, each glucosidic linkage broken by the action of β-amylase produces one new aldehyde group. The liberation of maltose as the sole reducing product resulting from β-amylatic action can be measured by any method suitable for aldehyde determination.

b. Decrease in Viscosity or Liquefaction of a Starch Paste. Unlike during α-amylatic degradation, there is only a very slow decrease in the molecular weight of the substrate during β-amylatic action, yielding practically no signs of liquefaction of a starch paste nor of viscosity reduction, while the reducing power increases noticeably.

c. Change in the Iodine-Staining Properties. β-Amylatic breakdown of amylopectin results in a very slow shift of the absorption maximum of the iodine-substrate complex to lower wavelengths. The iodine color of amylopectin or starch does not disappear as during α-amylatic degradation, but turns to purple. When amylose is the substrate, the picture is frequently troubled by the retrogradation of the substrate resulting in water-soluble but enzyme-resistant amylose, which stains blue with iodine and persists for a long time, sometimes until the end of the reaction, thus delaying or even preventing the disappearance of the iodine-staining capacity. In the absence of retrogradation, however, β-amylatic breakdown of amylose results in a gradual decrease in the intensity of the blue iodine color, followed by its complete disappearance.

d. Turbidity. There is no important decrease in the turbidity of glycogen solutions during β-amylatic digestion.

e. Change in Optical Rotation. One of the most characteristic properties of β-amylase is the Walden inversion that accompanies the hydrolysis. Thus β-maltose is formed during the enzymatic reaction, and consequently there is an upward mutorotation (*195a*).

2. *Nomenclature*

The liberation of the reducing sugar in the β-configuration is the reason why Kuhn termed this enzyme β-amylase, in contrast to α-amylase, which liberates the reducing groups in the α-configuration (*20*). Since the most characteristic property of β-amylase, besides producing the Walden inversion, is the liberation of reducing sugar, Ohlsson called it saccharogenic amylase (*21*, *22*).

3. *Mechanism of β-Amylase Action*

β-Amylase is capable of splitting only the penultimate α-1,4-glucosidic linkage from nonreducing chain ends (*21*, *22*, *26*, *121*, *195b*), thus liberating maltose as the sole reducing sugar. Maltose appears in the downward rotating β-form because of the Walden inversion occurring simultaneously with the cleavage of the bond. After the removal of one molecule of β-maltose from the end of the chain, the latter is again available to further attack by β-amylase. In this way, the substrate is broken down step by step from the nonreducing chain end until completion (amylose) or until the enzyme encounters an obstacle, such as a branching point or, in certain species of amylopectin, a phosphate ester group. The β-amylolytic breakdown of amylopectin or glycogen leaves behind, therefore, a limit dextrin of high molecular weight which is not further attacked by β-amylase alone. In conjunction with α-amylase or α-glucosidases, however, β-amylase may act further upon limit dextrin because new nonreducing α-1,4-glucosidic chain ends have been created by those enzymes. Thus, the action of α-amylase supplements or potentiates that of β-amylase.

The degradation of amylose, maltohexaose, or maltotetraose (*195c*) by β-amylase yields 100% maltose. Failure to arrive at complete degradation has frequently been reported in the literature. It may be due either to contamination by branched polysaccharides or, more likely, to the retrogradation of amylose. The latter phenomenon can be eliminated by repeated rejuvenations (*37*). Peat *et al.* (*196*) claim that amylose contains a certain number of bonds different from α-1,4-glucosidic linkages. According to these authors, β-amylase action is arrested by those undefined linkages, while the so-called Z-enzyme is believed to split the unknown bonds.

4. *Distribution of β-Amylase*

The most important sources of β-amylase are higher plants. The occurrence of this enzyme in microorganisms is much rarer than that of α-amylase, and β-amylases from animal sources are unknown.

a. *β-Amylase in Higher Plants*

(*1*). *Cereals.* Although cereal malts, in particular barley malt, are known to contain both α- and β-amylases, ungerminated grains of barley, wheat, rye, and, in lesser amount, oats have been known to contain practically only β-amylase (*197*). Traces of α-amylase in ungerminated cereals are thought to originate from the microflora on these grains (*114*). According to Kneen *et al.* (*120, 198*), the β-amylase content of cereal grains depends both on variety and on environment of growth. Thus, a given sample of wheat may have a higher β-amylase content than a certain sample of barley, or vice versa; on the average, however, barley grain tends to have more β-amylase than wheat grain. Six-row barley is generally richer in amylase than two-row barley (*199*).

The fate of β-amylase during the processes of ripening and germination has been frequently discussed. The amount of this enzyme in wheat is believed to increase during the short period of ripening and then to decrease again because β-amylase is partially transformed into an inactive "latent" form (*115*). The latter process appears to be, however, a mere combination of the enzyme with insoluble proteins, thus rendering β-amylase less soluble during this stage. During germination, the activity of β-amylase of an aqueous extract of barley increases two- to threefold because of proteolytic liberation of this enzyme from the latent state, while its total amount does not increase materially (*118, 200, 201*).

The distribution of β-amylase within the wheat grain has been studied by Linderstrøm-Lang *et al.* (*202, 203*). No β-amylase was found in the aleuron cells, but much in the subaleuronic layer. The amylase content in the endosperm is fairly low, increasing outward.

Ungerminated grains of corn (maize), millet, sorghum, and rice and their malts are practically devoid of β-amylase (*117*).

(*2*). *Higher plants other than cereals.* Rich sources of β-amylase occur in ungerminated soybean seeds (*204, 205*) and in sweet potatoes (*Ipomoea batatas*) (*206*); from the latter source this enzyme has been obtained in the pure and crystalline state (*207*). Furthermore, cabbage has been found to contain amylases of the β-type (*208*), and maple and birch saps have been reported to exhibit β-amylatic activity associated with α-amylase (*133*); later reports of the same author stress more the existence of α-amylase in these sources (*132*).

b. β-Amylase in Microorganisms. Certain organisms of *B. subtilis*, usually isolated from plant material, are believed to contain β-amylase together with α-amylase. Beckord and associates (*138, 209*) found that a bacterial isolate, after cultivation on wheat bran moistened with phosphate, developed an amylase different from commercial α-amylase preparations of *B. subtilis*. Though it dextrinizes starch and, therefore, contains α-amylase, its saccharifying capacity is higher than that of commercial bacterial α-amylase or of malt α-amylase. The authors concluded that the extract contains a mixture of α- and β-amylases, and they have actually shown different pH optima for dextrinizing and saccharifying activity. The possibility of the presence of a phosphorylase has not been ruled out.

β-Amylase in molds has been reported to occur in *Rhizopus japonicus* (*158*). These findings are based mainly on different pH optima of the extract for the liquefaction and the saccharification of starch.

5. *Properties of and Comparison between Various β-Amylases*

The β-amylases from two different sources, i.e., sweet potato (*207*) and barley malt (*210*), have been obtained in the pure and crystalline form; the properties of β-amylases from other cereals have been reported to resemble closely those of barley malt β-amylase (*197*), with the exception perhaps of differences in the heat stabilities. The latter property must be considered, however, as being largely conditioned by environmental factors, rather than as a representative characteristic of the enzyme. Thus, metal ions and certain proteins, present in crude or partially purified enzyme extracts, may act as heat stabilizers, as has been observed with bacterial α-amylase (*8, 193*). β-Amylases from bacterial and mold origin have not been characterized in detail, with the exception of their pH optima of action.

The properties of the two pure β-amylases are listed in Table XVI and can be compared to the corresponding data for α-amylases in Tables IX–XIII. The most important property of β-amylase, which permits differentiation of this enzyme from α-amylase, is, of course, its high ratio of saccharifying to dextrinizing activity; this ratio is more than six times higher for β-amylases than for the pure α-amylases (Table IX). The pH optimum of action, though considerably lower than that of α-amylases from nonanimal origins, does not appear to be significantly different from cereal α-amylases (Table X).

A very obvious difference between barley malt α- and β-amylases can be visualized by the so-called Wijsman agar plate test (*215*), which is based upon the difference in the diffusion constants of the two enzymes. β-Amylase, when applied together with α-amylase as a spot in the center of an agar plate containing soluble starch, diffuses faster than α-amylase, thus leaving a circular field in the center of the plate where the starch was broken down by both enzymes. This central field is surrounded by a ring where β-amylase alone was acting on the substrate. Upon developing the plate with iodine, a colorless central area (combined α- and β-amylase action) is separated from the outer dark blue field (no enzyme action) by a purple ring (β-amylase action).

Further distinction between cereal α- and β-amylases is possible because of the higher stability of β-amylase at low pH (*216*) and because of the higher heat stability of α-amylase (*21, 22, 122*). Thus, β-amylase activity will be eliminated from a mixture of the two enzymes by 20 minutes' heating to 70° with no appreciable loss of α-amylase, and storage at pH 3.5 in the cold will selectively remove the α-amylase.

TABLE XVI

Properties of β-Amylases

Property	β-Amylase from	
	Sweet potatoes (*211*)	Barley malt (*212, 213*)
Ratio of saccharifying to dextrinizing activity (*185*)	61	62
Specific activity per mg. protein	378 (*42*)	235
pH Optimum of activity	4–5	5.2
Activation energy (cal./mole)	—	16,200 (0–20°C.); 5530 (20–55°C.)
Turnover number	250,000	
Activation by Cl^- or Ca^{2+}	—	—
Solubility	—	15% at 2° at pH 7 in water (*42*); 4.5% in 0.24 sat. $(NH_4)_2SO_4$
Electrophoretic mobility at pH 7.9, $\mu = 0.1$ (cm.^{2} volt^{-1} sec.$^{-1}$)	—	2.4×10^{-5} (*42*)
Isoelectric point	4.74–4.79	6.0 (*42*)
Diffusion constant (cm.^{2} sec.$^{-1}$)	5.77×10^{-7}	—
Sedimentation constant (*S*)	8.9×10^{-13}	—
Molecular weight	152,000 ± 10%	—
pH Optimum of stability	—	4–8
% N	15.1	14.1 (*42*)
Essential groups	—SH	—SH
Arginine (%)	6.0 (*214*)	—
Tyrosine (%)	7.0 (*214*)	—
Cysteine + cystine (%)	0.79 (*214*)	—
Methionine (%)	4.32 (*214*)	—

Barley β-amylase is inhibited by nitrous acid and reactivated by hydrogen sulfide (*217, 218*). It is also inactivated by *o*-iodosobenzoate while 75% of the sulhydryl groups remain still titratable, and diffusion and sedimentation velocities remain unchanged (*219*). These workers conclude that the oxidation of —SH to —S—S— groups follows an intramolecular mechanism. Inhibition by ascorbic acid of sweet potato β-amylase (*220*) and of cereal β-amylase (*221*) is noncompetitive in contrast to that by *p*-chloromercury benzoate, suggesting that ascorbic acid inhibition follows a mechanism different from the reaction with sulfhydryl groups.

The properties of purified β-amylase from soybeans have been studied and found to be similar to those of the crystalline enzyme from sweet potato (*222*).

6. *Conclusions on β-Amylases*

In contrast to the wide distribution of α-amylases which are found in practically all animals and plants, the occurrence of β-amylase is strictly limited to a few microbial and vegetable origins. The latter are soybean seeds, sweet potato tubers, and cereal grains, as well as their malts; but by no means does β-amylase occur in all cereals. It appears that the presence of this enzyme follows no specific patterns and its absence in certain plants has no known harmful effect on their physiological functions. What role then does β-amylase play in the degradation of starch by plants? Unlike α-amylase, it does not attack native starch granules, but it enhances and supplements the action of α-amylase.

An interesting aspect of the role of β-amylase is its interrelationship with the phosphorylase system. Such a viewpoint has been clearly demonstrated by Porter's work (*223*). This author noted a considerable inhibition of plant phosphorylase by β-amylase, an effect which appears to be threefold. There is degradation of the polysaccharide synthesized from glucose-1-phosphate by phosphorylase. Secondly, the primer carbohydrate necessary to initiate the polysaccharide synthesis by phosphorylase is partially destroyed, resulting in a slowdown of polysaccharide synthesis. And finally, β-amylase appears to exert an inhibitory action upon phosphorylase which is independent of the two effects upon carbohydrate. Even more interesting is the observation by the same author that the presence of β-amylase changes the type of polysaccharide synthesized by phosphorylase from glucose-1-phosphate. Thus, in the presence of low concentrations of glucose-1-phosphate, a polysaccharide was obtained which stained red-purple with iodine when phosphorylase action was primed with achroic dextrins. A lengthening of the polysaccharide chains to produce a blue-staining polymer could be obtained by two different ways. First, by further addition of glucose-1-phosphate to the digest, and second, without additional glucose-1-phosphate but with the addition of β-amylase in amounts that were partially inhibitory.

A purely hypothetical but interesting interpretation of the above observations would be a possible mechanism of regulating the synthesis of the starch polysaccharides in preventing amylopectin from becoming glycogen. The latter polysaccharide, which has a higher degree of branching and contains shorter branches with uninterrupted sequences of α-1,4-glucosidic bonds than amylopectin, occurs almost exclusively in animals; i.e., glycogen is always synthesized in the absence of β-amylase. The less-branched amylopectin, however, containing longer α-1,4-glucosidic chains than glycogen, and the unbranched amylose with very long α-1,4-glucosidic chains are exclusively synthesized in plants. It appears logical to assume, therefore, that β-amylase occurring only in plants, like starch, may in some way be connected with the less frequent occurrence in starch than in glycogen of the α-1,6-glucosidic branching points. It is realized, however, that other mechanisms for the regulation of the degree of branching in the starch polysaccharides must exist.

Inasmuch as a comparison between the two pure β-amylases is permitted on the basis of the few data available, it appears that their action on the substrate is identical, as seen from the close agreement between their ratios of saccharifying to dextrinizing activity (see Table XVI). The chemical composition of the cereal enzyme differs, however, from that of the tuber enzyme; the nitrogen contents, the isoelectric points, and the values for specific activity per protein differ markedly for the two enzymes.

C. α-Glucosidases

In contrast to the amylases, which produce appreciable amounts of glucose only during the final stages of starch and glycogen breakdown, the enzymes that liberate glucose as the initial and sole product of hydrolytic action on these polysaccharides are generally called α-glucosidases. Because of their common specificity for the starch polysaccharides, this class of enzymes is also frequently referred to as amyloglucosidases. α-Glucosidases that split low-molecular oligosaccharides, e.g., the maltose-hydrolyzing enzyme maltase and others, are rather similar to the amyloglucosidases; they frequently occur together with them, and some of them are able to attack polysaccharides as well as oligosaccharides, so that a clear distinction between amyloglucosidases and α-oligoglucosidases is not always possible.

α-Glucosidases may be divided into three classes, i.e., those that break only α-1,4-glucosidic bonds, α-glucosidases specific for α-1,6-glucosidic linkages, and enzymes that may hydrolyze either type of bond. In many instances the specificity of the enzyme for one or the other of these two bonds has not been exactly established; other α-glucosidases are actually able to hydrolyze both 1,4- and 1,6-glucosidic linkages, and some α-glucosidases, though probably specific for only one type of bond, may occur in common with a glucosidase specific for the other type, and neither enzyme may have been sufficiently purified to permit of deciding to which class they actually belong. The following classification is, therefore, only a tentative one.

1. *α-1,4-Glucosidases*

A glucosidase was detected in the mold *Rhizopus delemar* (*224*) and has been isolated in highly purified form, free of α-amylase (*225*). It was called *glucamylase* and has been found to convert corn amylopectin to 95% theoretical glucose; glycogen to 92% glucose; residual β dextrins from amylopectin to 89% glucose, and maltose to 100% glucose (*226*). Since α-amylase is absent from the preparations of glucamylase, glucose cannot result from the action of maltase on degradation products of a primary reaction, but must itself be the primary product of hydrolysis by this enzyme. The authors believe that glucamylase, like β-amylase, attacks the nonreducing ends of the glucosidic chains of its substrate, and they conclude that this enzyme either hydrolyzes α-1,6-glucosidic bonds as well as α-1,4-glucosidic linkages or bypasses them in some manner. Phillips and Caldwell are somewhat in favor of the latter hypothesis, however, because of the obvious relationship between the small amounts of the limit dextrins, remaining after exhaustive action, and the degree of branching of the substrates. Thus, glucamylase would be specific for α-1,4-glucosidic bonds. Nothing is known about the nature of the limit dextrins, which, if the above assumption were correct, would have to contain all or most of the α-1,6-glucosidic bonds of the original polysaccharide. No data are available on the action of this enzyme on dextran or on isomaltose.

Meeuse (*227*) reported an enzyme in molds (*Mucor rouxianus* and several *Rhizopus* species) that yields glucose directly from starch; it also contains maltase, and glucose formation may be due to the combined action of β-amylase and maltase.

Common maltase of various origins has frequently been reported to liberate glucose from starch. Since maltase is almost always accompanied in nature by α-amylase, the reported action is likely to be due to the combined effect of the two enzymes; maltase alone is supposedly an oligosaccharidase and does not attack polysaccharides beyond a certain degree of polymerization. Maltase is extremely widespread in both animal and vegetable kingdoms, and its occurrence and specificity have recently been discussed in detail (*228*). In many instances, maltase supplements the action of the amylases, e.g., in the digestive tract of higher animals where it occurs in the intestinal flora and is believed to be responsible for the formation of glucose.

2. *α-1,6-Glucosidases*

An enzyme of animal origin belonging to this class is the amylo-1,6-glucosidase prepared by Cori and Larner (*229*) from rabbit muscle. These authors were able to show that amylo-1,6-glucosidase liberates glucose from glycogen limit dextrins (prepared by exhaustive action of muscle phosphorylase on glycogen), but not from native glycogen. This action consists in the hydrolytic splitting of the α-1,6-glucosidic bond of the branching point after the latter had been exposed by the exhaustive action of phosphorylase. The physiological significance of this enzyme is easily understood from its ability to supplement phosphorolytic breakdown of glycogen. Thus, a mixture of these two enzymes is able to digest glycogen to completion, whereas exhaustive action of phosphorylase alone yields only 36% degradation. In amylopectin the branching points are hydrolyzed in the same way by amylo-1,6-glucosidase, after being exposed by phosphorylase action. The amount of free glucose liberated from a branched polysaccharide by the exhaustive action of a mixture of phosphorylase and amylo-1,6-glucosidase has been found to be directly proportional to the number of branching points of the polymer. This fact has been applied to end-group determinations of glycogens and amylopectins from various origins, with the assumption that the number of nonreducing end groups is practically equal to the number of branching points in a polysaccharide of sufficiently high molecular weight. Amylo-1,6-glucosidase has also been found to hydrolyze isomaltose, but this reaction appears to be very slow (*229*). The study of the histology and cytochemistry of human skin has resulted in the demonstration of amylo-1,6-glucosidase in stratum spinosum, in growing hair follicles, and in the sebaceous glands (*230*).

In plants, an enzyme has been detected which catalyzes the hydrolytic scission of α-1,6-glucosidic linkages in amylopectin (*231*). It has been isolated from the potato and the broad bean and is known as R-enzyme. Its action is strictly that of a "debranching" enzyme and it appears to have no action on the α-1,4-bonds.

An oligo-1,6-glucosidase that splits α-1,6-glucosidic bonds in low molecular weight dextrins only and is different from R-enzyme has been found in intestinal mucosa (*232*).

3. *Unspecific α-Glucosidases*

In 1940, a glucosidase from yeast was reported by Meyer and Bernfeld (*233*). This enzyme has been found to split the terminal α-1,6-glucosidic bonds of limit dextrins prepared by exhaustive degradation of corn amylopectin by β-amylase. The molecular weight of the limit dextrin was not significantly reduced by the glucosidase action. After treatment of limit dextrin with yeast glucosidase and subsequent heat inactivation of the latter enzyme, the polysaccharide had become again susceptible to β-amylase action. Thus, a second limit dextrin was obtained which still stained with iodine. In this manner, alternate degradation of limit dextrin by β-amylase and by yeast glucosidase led to a progressive, step-by-step degradation of the substrate, which maintained its iodine staining capacity and its macromolecular properties during several steps. This stepwise degradation of amylopectin by the two enzymes was the basis that led Meyer and Bernfeld to advance the bushlike structure of amylopectin (*233*).

The yeast α-glucosidase has not been sufficiently purified to claim its specificity for 1,6-bonds alone. Evidence to prove or to disprove the ability of this enzyme to split α-1,4-glucosidic bonds is not available.

The existence of α-1,6-glucosidases in higher plants (barley malts) has been demonstrated by Kneen (*127*, *234*) and Schwimmer (*235*), while Kerr and co-workers (*236*, *237*) have shown a similar enzyme to occur in molds. The enzyme was called "limit dextrinase" because of its ability to make limit dextrins available to saccharification by β-amylase, though the mechanism of their action is not completely established. Although it is not known whether barley limit dextrinase action is confined to the 1,6-glucosidic bonds, a mold amyloglucosidase from *Aspergillus niger* has been shown to break down amylose and amylopectin, as well as limit dextrins (*238*) and, thus, to hydrolyze both types of bonds. The action of a limit dextrinase from *A. niger* on dextran was found to be extremely slow, though isomaltase was hydrolyzed rapidly (*239*). A crystalline preparation of limit dextrinase from *A. oryzae* has been obtained by Underkofler and Roy (*166*).

Another enzyme that appears to be capable of hydrolyzing both types of bonds is the maltase of *Clostridium acetobutylicum*, first described by Hockenhull and Herbert (*154*). The action of this enzyme was found by French and Knapp (*153*) to consist in the hydrolytic removal of individual glucose units from the nonreducing ends of amylopectin, amylose, or oligosaccharide. It is capable of hydrolyzing at least the majority of the branch linkages, resulting in extensive hydrolysis of amylopectin, amylose, or dextrins to glucose.

Black-*koji* molds (*Aspergillus usanii*) have been found to contain a "debranching" enzyme (*240*), which has not been further defined.

4. *Conclusions on α-Glucosidases*

The physiological significance of α-glucosidases lies obviously in the tendency of the organism to break down starch or glycogen to the level of glucose, which, in animals, is more rapidly absorbed through the cell walls and which is essential for carbohydrate metabolism.

There appears to be no significant difference between the end products of the combined action of α-amylase and maltase on starch taking place in the intestinal tracts of animals and the direct action of glucamylase alone. The action of the latter enzyme represents an alternate pathway used by lower organisms; it achieves the same task and yields the same final products.

Mammalian α-1,6-glucosidase (amylo-1,6-glucosidase) is necessary to supplement phosphorylase action during its degradative phase, whereas the other enzymes specific for α-1,6-bonds apparently serve to remove the obstacles, encountered by the amylases in the form of branching points in amylopectin. Here again, there appears to be no significant difference in the end products whether the glucosidase acts upon the high molecular substrate and achieves the degradation alone or whether it is an oligosaccharase, called oligo-1,6-glucosidase, of the type found in the intestinal mucosa, which acts in conjunction with amylases. Apparently, the higher organisms prefer the pathway in which high molecular substrates are broken down by a first enzyme to low molecular weight dextrins which in turn are broken down further by a second set of enzymes, as in the amylase-maltase, and the amylase-oligo-1,6-glucosidase, systems. Lower organisms appear to have the tendency of seeking alternate pathways using a single enzyme only, such as glucamylase, or the maltase of *C. acetobutylicum.*

III. Dextranase

Dextran is a bacterial polysaccharide produced by certain microorganisms when grown on a sucrose-containing medium. It has many analogies with amylopectin (or glycogen); both polysaccharides have branched structures and are built up from D-glucose units joined together by the same types of bonds, i.e., α-1,4- and α-1,6-glucosidic linkages. The proportion in which the two types of bonds occur in these polysaccharides is reversed, however. Thus dextran contains mainly α-1,6-glucosidic linkages, which make up the main chains, while the branching points are of α-1,4-glucosidic and, in some cases, of the α-1,3-glucosidic nature. In spite of the fact that amylopectin and dextran contain the same type of bonds, their biological degradation is carried out by completely separate enzyme systems. Amylases are known not to hydrolyze any bonds in dextran (*241*), and limit dextrinase action on dextran has been found to be extremely slow (*239*), although isomaltose was hydrolyzed rapidly by the latter enzyme.

Hydrolytic enzymes that digest specifically dextran have been recognized during the past ten years, and their investigation has provided considerable insight into the nature and action of dextranases.

A. Mechanism of Action

Early experiments (*242*, *243*) with dextranase from cultures of bacteria and molds demonstrated the ability of this enzyme to reduce the viscosity of dextran solutions and, hence, to act in the general manner of an α-amylase. Mold dextranase was reported to split the α-1,6 bonds of dextran at random, and to stop at a degradation of 50–60% isomaltose (*244*).

The products of degradation of dextran by dextranase from two strains of *Penicillium funiculosum* have been studied by Jeanes *et al.* (*245*). After treatment of dextran with the enzyme from strain 1768, these authors found among the end products 3.7% glucose, 45% isomaltose, and 22% isomaltotriose, whereas dextranase from strain 1132 produced slightly different end products, i.e., 11% glucose, 53% isomaltose, and 7% isomaltotriose.

The action of mammalian dextranase was reported, however, to yield glucose as the sole product of dextran degradation (*246*, *247*), and Sery and Hehre (*248*), working with dextranase from intestinal bacteria, also found glucose as the sole and major product of dextran breakdown, but observed different pH optima for the liquefying and saccharifying action of this enzyme. These workers concluded that two separate enzymes are involved in the biological degradation of dextran, the action of which would be analogous to that of α- and β-amylases.

B. Occurrence

Dextranase appears to be quite widely distributed in microorganisms (molds, bacteria), but its presence in mammalian tissues has equally been reported. Like amylases and cellulases, dextranase is an extracellular enzyme. A summary of dextrinase occurrences is given in Table XVII.

C. Conclusions

Although the types of glucosidic linkages in the starch polysaccharides and in dextran are the same, i.e., α-1,4- and α-1,6-glucosidic bonds, the enzymes that digest the two groups of polysaccharides are completely different. This implies that the specificity of these enzymes depends not only on the nature of the glucosidic bond, but is governed also by the type of the adjoining glucosidic bonds and/or by the structure of the whole polysaccharides.

TABLE XVII
Occurrence of Dextranase

	Source	References
Animals	Liver and spleen of bulls, rabbits, dogs	(*246*, *247*, *249*)
Bacteria	*B. cellvibrio fulva*	(*242*)
	Bacteroides in human stools	(*248*, *250*)
Molds	*Penicillium lilacinum* Thom	(*243*, *244*, *251*)
	P. funiculosum Thom	(*243–245*, *251*)
	P. purpurogenum	(*244*)
	Verticillium coccorum Westerdijk	(*243*, *251*)
	P. verruculosum	(*252*)
	Spicaria violacea	(*252*)

Though the substrate dextran appears to be formed exclusively in bacteria, the presence of dextranases in animal tissues (spleen, liver) and in human stool bacteria is significant. The presence of dextranase in stool bacteria may be an adaptive mechanism destined to the elimination from the animal organism of dextran carried along by dextran synthesizing microorganisms, but the origin and role of dextranase in spleen and liver remain unexplained. The possibility that this source of dextranase may also be due to bacterial fauna cannot be entirely neglected, however. The complete absence of dextranase in higher plants where the introduction of dextran-producing microorganisms is less likely than in animals, strengthens the latter hypothesis.

IV. Cellulases

A. The Substrate

Cellulose is found in nature in association with lignin as the structural substance of the cell walls of plant tissue. Its highly insoluble nature gives cellulose a particular position in its role as a substrate for cellulases. Undoubtedly, certain analogies exist between the very slow attack of the β-1,4-glucosidic chains in native cellulose fibers by cellulases, and the degradation of α-1,4-glucosidic bonds in retrograded amylose by α-amylase which, although very slow, nevertheless is measurable.

Various materials have been used as substrates for the practical purpose of measuring and identifying cellulase action. Karrer (253) compared the influence of a number of substrates, such as cotton, filter paper, mercerized cotton, regenerated cellulose trinitrate in which the nitrate groups had been removed by treatment with alcoholic ammonium sulfide, cellulose that had been reprecipitated from a xanthate solution (viscose) or from a solution in cuprammonium hydroxide, and alkali-soluble celluloses obtained from the triacetate or after treatment with hydrochloric acid. In addition, hydrocellulose and swollen cellulose fibers, obtained by the action of phosphoric acid (*254*), have been used as substrates. Recently, substituted cellulose derivatives, such as carboxymethylcellulose (CMC), carboxymethylhydroxyethylcellulose (CMHEC), hydroxyethylcellulose (HEC), sulfoethylcellulose, and cellulose sulfate (*255*), have been introduced for the assay of cellulose activity (*256, 257*), which thus can be measured by the decrease in viscosity of the substrate solution rather than by determining the increase in reducing power, as was necessary with the other substrates.

B. MECHANISM OF ACTION

At least three different groups of enzymes are known to be involved in the biological breakdown of cellulose. The first group of cellulases, called C_1 enzyme by Reese and Levinson (*258*) is believed to initiate the attack of cellulose in fibers, resulting in a rapid loss of their tensile strength. Enzymes of this kind have been reported to occur in certain fungi (*Chaetomium globosum*), in which they are apparently not accompanied by other cellulases (*259*). The substrate, thus modified, or partially soluble cellulose, hydrocellulose, or carboxymethylcellulose, is then hydrolyzed by the C_x enzyme into water-soluble fragments of low molecular weight. The latter enzyme, being the cellulase proper, is believed to split the chains at random, and the mechanism of its action resembles closely that of α-amylase. Finally, β-glucosidases such as cellobiase continue the breakdown to glucose, which has been found to be the sole reducing product of exhaustive biological cellulose degradation (*260*), and it has been reported by Karrer (*253*) that the conversion of cellulose to glucose may exceed 93%.

Although the β-glucosidase usually occurs in association with the cellulases (C_x), these two enzymes have been separated from one another by the adsorption of the β-glucosidase on aluminum metahydroxide at pH 3.5 and subsequent elution in slightly alkaline medium (*261*). The same authors have shown that the cellulase fraction is distinctly specific for high molecular substrates only, whereas the β-glucosidase attacks mainly the low molecular weight cellodextrins, as seen from the data in Table XVIII. It is also evident that the β-glucosidase fraction is not exclusively a cellobiase, i.e., an enzyme specific for the disaccharide only, but that it is capable of hydrolyzing also oligosaccharides up to the hexaose, and possibly even of higher degrees of polymerization.

Festenstein (*262*) has shown that cellobiase from sheep-rumen microorganisms is completely inhibited by 2.5 m*M* gluconolactone, whereas the cellulase from the same origin, acting on carboxymethylcellulose, is only partially inhibited at this concentration of the lactone. When cellobiase activity is completely inhibited, only traces of low molecular cello-oligosaccharides (cellobiose, cellopentaose) are detectable by chromatography, whereas glucose is the predominant product of hydrolysis in the absence of inhibitor.

TABLE XVIII

ENZYMATIC HYDROLYSIS OF CELLULOSE DEXTRINS BY CELLULASE C_x AND β-GLUCOSIDASE[a]

Substrate	% Glucosidic bonds hydrolyzed in 24 hours	
	Cellulase fraction (C_x)	β-Glucosidase fraction (cellobiase)
Hydrocellulose	7.3	1.3
Cellodextrin	34.0	2.6
Cellohexaose	17.4	31.1
Cellotetraose	9.0	31.4
Cellotriose	0.6	34.7
Cellobiose	0	77.0
Methyl β-glucoside	1.4	16.1

[a] Calculated from data of Grassmann *et al.* (*261*).

Cellulase from culture filtrates of the mold *Myrothecium verrucaria* has been found to withstand prolonged boiling (*263*); it thus can easily be freed from accompanying cellobiase. The end products of the action of the heat-treated cellulase are mostly cellobiose and glucose, together with small amounts of tri-, tetra-, penta-, and hexaoses; cellotriose is also split into glucose and cellobiose. These observations indicate that the cellulose chains are split at random by cellulase by a mechanism similar to the action of α-amylase on amylose.

The influence of substitution of the substrate on the enzymatic degradation of cellulose has been studied by Reese (*255*). It has been suggested that two unsubstituted glucose units must adjoin for the enzymes to act; minimum degrees of substitution of cotton for protection against enzyme action are shown in Table XIX.

Attempts at purification of cellulases have clearly demonstrated the multiple nature of this enzyme system. Zone electrophoresis of fungal cellulase yielded at least eight components with C_x activity and different electrophoretic mobilities (*264*). Paper and column chromatography (*265*, *266*), as well as paper electrophoresis (*267*), have equally shown the occurrence of several components.

TABLE XIX

MINIMUM DEGREES OF SUBSTITUTION (DS) OF COTTON TO PROTECT FROM CELLULYTIC ACTION (*255*)[a]

Fabric treatment	Minimum DS for protection
Methylation	0.7
Acetylation	0.5–0.7
Cyanoethylation	0.5
Formaldehyde	0.2–0.3
Phosphate and urea	0.25
Phenylhydrazine	0.004

[a] Reproduced by courtesy of *Industrial and Engineering Chemistry.*

It has been observed that lignin inhibits the bacterial degradation of cellulose (*268*). Thus, the effect of cellulases on wood is frequently much smaller than on pure cellulose. In addition, the compact nature of wood is, of course, a limiting factor for the enzymatic accessibility, and it has been observed that grinding of wood increases its bacterial degradation (*269*, *270*).

C. OCCURRENCE

Cellulases have been found in both animal and vegetable kingdoms. They are present in microorganisms and in higher plants; their occur-

TABLE XX

OCCURRENCE OF CELLULASES IN THE ANIMAL KINGDOM

	Source	References
Insects	Wood-eating insects (*Stromatium fulvum*), in gastric juice of larvae	(*271*)
Mollusks	Snails (*Helix pomatia*), in gastric juice	(*253*, *272–275*)
	Snails (*Helix aspersa*), gastric juice	(*276*)
	Earthworms (intestine)	(*277*)
	Shipworms (*Teredos*)	(*278*)
	Clams (crystalline style)	(*279*)
Protozoa	Amebas fed on soil bacteria	(*276*)
	Intestinal flagellates of termites and roaches (*Trichomonas*, *Termopsidis*)	(*260*)
	Cattle rumen protozoa (*Eudiplodinium*)	(*280*)

rence in animals appears to be limited to mollusks and protozoa. The occurrence of cellulases has been discussed recently by Pigman (*254*) and is summarized in Tables XX and XXI.

TABLE XXI

Occurrence of Cellulases in the Vegetable Kingdom

Source	References	Source	References
Higher plants		Molds	(*284*)
Unsprouted barley	(*281*)	*Aspergillus fumigatus*	
Barley malt	(*281, 282*)	*Aspergillus terreus*	
Potato sprouts	(*283*)	*Chaetomium globosum*	
Bacteria	(*284*)	*Cladosporium herbarum*	
Cytophaga rubra		*Fusarium moniliforme*	
Cellulomonas biazotea		*Memnoniella echinata*	
Corynebacterium sp.		*Myrothecium verrucaria*	
Cellvibrio vulgaris		*Penicillium luteum*	
Sporocytophaga myxococcoides		*Stachybotrys atra*	
		Trichoderma viride	
Actinomycetes	(*284*)		
Streptomyces cellulosae			

At least seventy species of bacteria, molds, and actinomycetes have been found to split cellulose; they are reviewed in detail by Siu (*284*). Only the most important representatives of the abundant list of cellulose splitting microorganisms are listed in Table XXI.

D. Conclusions

The overwhelming abundance of cellulase in microorganisms and its rather spotty appearance in higher plants and in the animal kingdom cannot but create the impression that those few occurrences may actually be due to the growth of certain microorganisms at the sites where cellulase has been detected in animals and higher plants and that the origin of cellulases may be limited to bacteria, molds, actinomycetes, and, possibly, protozoa.

That the ability to digest cellulose by wood-eating insects is actually due to the intestinal protozoa and not to enzymes secreted by the animal itself, appears to be well established. Similarly, the digestion of cellulose in the rumen of cattle has been found to be the task of protozoan enzymes, rather than of tissue enzymes.

It has not been determined whether the biological degradation of cellulose to glucose is achieved by a single or by several enzymes and what are the functions of the multiple cellulases from the same source.

V. Enzymes That Hydrolyze Other Polyglucosans

A. Lichenase

Lichenin is the polysaccharide of lichens and is similar in structure to cellulose. It is distinct from the latter, however, by the presence in it of about 25% of β-1,3-glucosidic linkages, probably distributed at random throughout the molecule.

Whether the biological degradation of lichenin is actually due to enzymes different from cellulase, and whether cellulase is able to attack lichenin, is not exactly known. It is obvious, however, that the action and, in particular, the occurrence of lichenase are similar to those of cellulase. Pigman (*254*), in reviewing this enzyme, indicates its presence in mammals (rabbit pancreas, cattle, and swine stomachs), in invertebrates (*Helix pomatia*, sponge, earthworm, starfish, mussel, crab, etc.), in higher plants (sprouted and dormant barley, wheat, rye, and oats and also corn, spinach, bean seeds, and hyacinth bulbs), in bacteria (*Cytophaga*), and in molds. Its abundance in the seeds of most plants has suggested that its substrate lichenin is more widely distributed in nature than commonly thought (*285*).

B. Laminarase

Laminarin is a polysaccharide of seaweeds composed entirely of D-glucose and is probably built up by β-1,3-glucosidic bonds. Its biological degradation by laminarase has been found to yield D-glucose, laminarobiose, and higher oligosaccharides (*286*). The enzyme has been found to occur in wheat, oat, barley, potato, and hyacinth bulbs. Its specificity does not appear to be limited to laminarin since it is also capable of splitting lichenin. No experiments with purified enzyme preparations have been published, and it cannot be decided whether laminarase is different from lichenase and/or cellulase.

VI. Enzymes That Hydrolyze Mannans and Mannogalactans

Because of the fact that the substrates for these enzymes have not been prepared in sufficiently purified form, only little information is available on the enzymes. The sources for these enzymes are again similar to those of cellulose (*254*), i.e., the gastric juice of the snail and other mollusks, plant seeds, malt extract, bacteria, and molds (*Aspergillus oryzae*). The possibility that mannan- and mannogalactan-splitting enzymes are identical with cellulase must be taken into consideration.

VII. Enzymes That Hydrolyze Fructans

A. Invertase

It has been reported that commercial invertase is capable of hydrolyzing, although rather slowly, so-called glucofructans. Thus, glucofructans from *Asphodelus,* from daffodils, from *Lycoris* (*287*), as well as two polysaccharides from *Allium* (*288*), when incubated with invertase preparations, undergo a change in their optical rotation which, though negative in the beginning, turns even more negative during enzymatic hydrolysis. The mutarotation of the digests is downward in the beginning and becomes upward toward the end of the reaction. The authors conclude that the downward mutarotation is due to the liberation of fructose whereas the upward mutarotation is the result of the liberation of α-glucose.

Fructans containing only fructose are also digested by invertase, and the fructan phlean isolated from timothy roots (*287*) as well as a fructan from *Allium ursinum* (*288*) behave in a similar fashion upon incubation with invertase, with the exception that the mutarotation is always upward because of the lack of liberation of α-glucose.

B. Inulase

Pringsheim *et al.* (*289, 290*) have isolated an enzyme from *Aspergillus niger* that is capable of hydrolyzing inulin to about 90%. They later found small amounts of glucose in the digests which contained mainly fructose (*291, 292*). Purified inulase preparations from two samples of bakers' yeast and, to a lesser degree, from three samples of brewers' yeast were obtained by Adams *et al.* (*293*). These authors noted the appearance of about 1.7% glucose during the hydrolysis which they ascribed to originate from inulin. The inulase preparations were not able to hydrolyze α- or β-dextrins, but contained small amounts of enzymes splitting low molecular weight β-glycosides.

VIII. Enzymes That Hydrolyze Pentosans (Xylanase)

Xylan together with other polysaccharides, such as mannogalactans and the so-called hemicelluloses, constitutes a group of plant polysaccharides which have been only incompletely separated from one another. Xylan is the best-defined substance of this group, and its biological degradation has been the subject of several publications. Again, the occurrence of xylanase is very similar to that of cellulase and has been reviewed by Pigman (*254*). This enzyme is found in mollusks (snails), insect larvae, higher plants (barley malt), and molds (*Aspergillus* *oryzae, A. niger*). Hydrolysis of xylan by this group of enzymes appears to be slow and incomplete. The insolubility of the substrate appears to be a limiting factor during xylan degradation, and this behavior is analogous to that encountered during enzymatic degradation of cellulose and of retrograded amylose.

IX. Enzymes That Hydrolyze Polyuronides

A. Pectic Enzymes

This group of enzymes has recently been reviewed by Kertesz (*294*). According to the complexity of their substrates, there are at least three distinct enzymes participating in the biological breakdown of pectic substances.

1. *The Substrate* (*295*)

a. Protopectin. The parent substance, protopectin, is insoluble in water, and its chemical nature is not yet completely understood. Protopectin is closely associated with cellulose in the matrix of the cell walls. Restricted hydrolysis of insoluble protopectin yields pectins or pectinic acids.

b. Pectinic Acids and Their Salts. Pectinic acids and pectinates are polygalacturonic acids in which from 20 to 90% of the carboxyl groups are present as methyl ester and which are capable of producing colloidal solutions in water.

c. Pectins. Pectins are chemically not different from the pectinic acids. They are capable, however, of forming jellies with sugar and acid under suitable and well-specified conditions.

d. Pectic acids and Their Salts. Pectic acids and pectates are polygalacturonic acids that are essentially free from methyl ester groups and capable of yielding colloidal solutions with water.

2. *Nomenclature of Pectic Enzymes*

According to the *chemical reactions* involved, there are at least two possible enzymes participating in the biological degradation of pectic substances. The first is an enzyme that causes the fissure of glycosidic linkages and, hence, reduces the molecular weight of the substrate. The second is an esterase which is capable of splitting the methyl ester groups. Various terms have been used for the enzymes fulfilling these two functions, and it has been postulated that more than one enzyme that splits the glycosidic bonds exists. Table XXII summarizes the functions and terminology of pectic enzymes.

The various terms for the active principle that catalyzes the hydrolysis of glycosidic bonds in polygalacturonic acids may all relate to one and the same enzyme, or there may be several enzymes involved in this process, e.g., one which might attack only the insoluble substrate protopectin (protopectinase), another which might produce only a partial depolymerization (depolymerase), and a third which catalyzes the breakdown to low molecular substances (pectinase). The splitting of the methyl ester bond is probably accomplished by a single enzyme, which has received several names.

TABLE XXII
FUNCTION AND TERMINOLOGY OF PECTIC ENZYMES

Function		
Hydrolysis of glycosidic bonds	Hydrolysis of methyl ester bonds	Name
×		Protopectinase
×		Depolymerase (DP) (*296*)
×		Pectin polygalacturonase (PG)
×		Pectinase
×		Pectolase
×		Polygalacturonase
	×	Pectin methylesterase (PM)
	×	Pectase
	×	Pectin methoxylase
	×	Pectin esterase

3. *Pectic Enzymes That Hydrolyze Glycosidic Bonds*

a. Methods of Activity Determination. In analogy to the determination of amylases, the activity of this group of enzymes can be determined by various changes in the properties of the substrate occurring during enzymatic hydrolysis. For the assay of pectin polygalacturonase (PG), pectinase, pectolase, and polygalacturonase, increase in reducing power, decrease in viscosity, change in optical rotation, and gradual disappearance of alcohol or calcium precipitating material have been extensively used, and these methods have recently been reviewed by Kertesz (*294*). The measurement of protopectinase is based upon the softening of water-insoluble tissue preparations of protopectin.

b. Occurrence. Like cellulase, and many other polysaccharidases acting on the insoluble material of cell membranes, pectolytic enzymes have been found in the digestive tract of the Roman snail (*Helix pomatia*) (*297*). This source, however, seems to be the sole occurrence of pectic enzymes in the animal kingdom, and it is not known whether the enzymes are actually secreted by the snail or formed by the fauna of the digestive tract.

In the vegetable kingdom, pectic enzymes have been found in higher plants, i.e., in tomatoes (*298, 299*) and in germinated barley (*300*), rye, oats, as well as in fruits, e.g., apples, pears, and peaches. Kertesz (*294*), however, states that "it appears that the enzyme might not be present in fruits as commonly as it was formerly assumed."

In lower plant life, pectic enzymes are very common. They occur in algae (Thallophyta) (*301*); in bacteria (*302*), e.g., in *Bacillus carotovorus, B. cannabinus, B. amylovorus, Pseudomonas solanacearum,* etc. (*303*); and in molds, e.g., in *Myrothecium verrucaria, Sclerotinia libertiana, Botrytis cinerea, Rhizopus tritici, Aspergillus oryzae, Fusarium chromiophthoron,* etc. (*302*).

c. Properties. The presence of methyl ester groups in protopectin, pectinic acids, and pectins appear to have an unfavorable influence on the action of the glycosidic linkages splitting pectic enzymes. Although pectic acids are most readily split, esterification of the carboxyl groups with ethylene oxide gradually reduces the enzymatic availability of the modified substrate, and completely esterified pectic acid glycolate is enzyme resistant (*304*).

Purified polygalacturonase was found to be highly specific (*305*); starch, maltose, sucrose, oxidized cellulose, carboxymethylcellulose, inulin, and ovomucoid were not measurably attacked by the enzyme. Even a large number of uronic acid-containing polysaccharides, such as gum arabic, gum ghatti, mesquite gum, gum tragacanth, alginic acid, and pneumococcus polysaccharide type I did not act as substrates, nor were methyl galacturonide and its methyl ester hydrolyzed by this enzyme. Mucopolysaccharides like hyaluronic acid and heparin were equally not attacked by the same enzyme preparations.

Polygalacturonases from molds are activated by many salts, especially by chlorides (*306*), and the activation power increases in the following sequence Li < Na < K < Rb < Cs. This property has certain analogies to the activation of animal amylases by sodium chloride.

4. *Pectic Enzymes That Hydrolyze Methyl Ester Bonds*

a. Methods of Activity Determination. The assay of pectinesterase can be accomplished either by the determination of the amount of methanol liberated (*307, 308*) or by direct titration of the increase in carboxyl groups (*309*). In both cases, pectinic acid serves as a substrate.

b. Occurrence. Pectinesterase has not been reported to occur in the animal kingdom, but this enzyme is widely distributed in higher plants and in molds.

Holden (*308*) measures its activity in the sap, the fibers, and the leaves of various plant species (see Table XXIII). Pectinesterase has been found in tobacco fibers (*310*), in the tomato fruit, in cherries, alfalfa, malt, and in many other higher plants (*302*).

TABLE XXIII

DISTRIBUTION OF PECTIN ESTERASE IN VARIOUS PLANT SPECIES[a]

Plant	Sap (pH)	Total activity in sap (%)	Activity (units/gm.)	
			Fiber	Leaf
Nightshade (*Solanum dulcamara*)	5–6	9	0–060	0–024
Tomato (*Lycopersicon esculentum*)	6–0	33	0–063	0–023
Potato (*Solanum tuberosum*)	6–2	16	0–069	0–015
Elder (*Sambucus nigra*)	6–3	8	0–050	0–016
Peppermint (*Mentha piperita*)	6–3	17	0–005	0–0016
Melilot (*Melilotus altissima*)	8–6	43	0–016	0–014
Clematis (*Clematis jackmanni*)	4–8	9	0–009	0–006
White bryony (*Bryonia dioica*)	6–3	16	0–022	0–011
Beaked parsley (*Chaerophyllum sylvestre*)	5–6	9	0–026	0–010
Rhubarb (*Rheum rhaponticum*)	4–3	15	0–007	0–0034
Lemon, peel (flavedo + albedo)	—	—	0–062 unit/gm. peel	
Orange, peel (flavedo + albedo)	—	—	0–073 unit/gm. peel	

[a] According to Holden (*308*). Reproduced by courtesy of the *Biochemical Journal.*

Pectinesterases are further known to occur together with polygalacturonases in various molds, especially in the species of *Mucor, Penicillium, Aspergillus, Botrytis,* and others (*311*).

Bacterial sources for pectinesterase have been found and investigated in *Pseudomonas solanacearum* (*303*).

c. Properties. A comparison of fungal pectin esterase with that of higher plants has revealed remarkable differences between the enzymes from these two origins (*299*). The pH optimum of the mold enzymes is between 4.5 and 5.5, but the pectin esterases from higher plants are barely active in this pH region. The latter enzymes are active at and above pH 7, depending on the salt concentration. Although tomato pectin esterase activity is hardly affected by the salt concentration, the fungal enzyme is almost twice as active in the presence of 0.1 M NaCl than without. Further distinctive marks between the two enzymes are found in their resistance to chemical inactivators, in particular detergents, in their temperature quotients, energies of activation, and thermal inactivation rates.

This difference between fungal and tomato pectin esterase is analogous in many respects to the differences between malt and mammalian α-amylases, in particular with regard to pH optima and NaCl activation.

5. *Conclusions*

The biological degradation of pectic substances is achieved by at least two different enzymes, possibly by a larger number; i.e., one that solubilizes the insoluble protopectin (protopectinase), a second that depolymerizes the substrate (depolymerase), a third (pectin esterase) that splits off methyl ester groups, and a fourth (polygalacturonase) that breaks down the remainder of the substrate to galacturonic acid. Whether protopectinase, depolymerase, and polygalacturonase are actually different enzymes cannot be ascertained on the basis of the information available. It appears that in many instances the enzymes occur together; they are found rarely in animals, frequently, however, in higher plants and in molds. At least one of the pectic enzymes (pectin esterase) has been reported to be of different nature whether it occurs in molds or in higher plants.

If it is true that protopectinase, depolymerase, and polygalacturonase are different enzymes, certain analogies of the pectic enzymes with the amylolytic enzymes become evident. Protopectinase, depolymerase, and polygalacturonase supposedly split the same type of glycosidic linkage in their substrate, and so do α-amylases, β-amylases, and α-1,4-glucosidases in starch. A second type of a chemical bond in the pectic substances (methyl ester groups) is hydrolyzed by a different enzyme (pectin esterase) and, in analogy to this, a second type of bond in amylopectin (α-1,6-glucosidic linkage) is hydrolyzed by an enzyme specific only for this bond (amylo-1,6-glucosidase).

There is no doubt that pectic enzymes together with cellulases play a major role in the solubilization of insoluble cell wall material of plant life. It is understandable, therefore, that these enzymes occur mostly in plants, in microorganisms, and, in isolated cases (cellulases), in the digestive tract of some animals, mainly herbivores.

Pectic enzymes were found not to play a major role in the wilting of plants invaded by certain bacteria (*303*). This process is obviously due to cellulytic enzymes alone.

B. ALGINASE

Alginic acid is a seaweed polysaccharide made up entirely of D-mannuronic acid units linked together to unbranched chains by β-1,4-bonds.

Alginase activity, as measured by the reduction of the viscosity of alginic acid solutions, has been reported in bacteria, where its occurrence is rare, however (*312*), and in the intestine of the sea urchin (*313*). In sea-water bacteria, 12 out of 200 strains investigated were found to be alginolytic (these bacteria are termed *Alginovibrio*), and 9 out of 200 soil bacteria fall into two genera, namely, *Alginomonas* and *Alginobacter*. Alginic acid being a seaweed polysaccharide, Thjøtta and Káss conclude that it is peculiar to find the most energetic alginase activity in soil bacteria, rather than in sea-water bacteria.

Studies on the specificity of alginase toward other polyuronides have not been published. It is known, however, that polymannuronate is not hydrolyzed by polygalacturonase (*305*).

C. Hemicellulases

Hemicellulose is a variety of plant polysaccharides that are water insoluble and occur in the cell walls in association with cellulose and pectic substances. This group of structural polymers comprises a large number of individual polysaccharides which all contain hexoses, pentoses, and glycuronic acids (*295*).

Enzymes that solubilize these hemicelluloses have been found in association with cellulases and pectic enzymes, in particular in the digestive tracts of the snail and shipworm, in potato sprouts, and in *Aspergillus oryzae* (*254*).

Because of the difficulties in obtaining the substrate in pure form, this class of enzymes is not well defined and delimited from cellulases, pectic enzymes, xylanases, etc., i.e., from enzymes which bring about the biological breakdown of insoluble cell wall material.

X. Enzymes That Hydrolyze Pneumococcal Polysaccharides

Among the large number of heteropolysaccharides known, only a few have been reported to possess specific enzymes that catalyze their degradation.

Pneumococcus polysaccharide type III, which consists of D-glucose and D-glucuronic acid in a 1:1 ratio, is hydrolyzed by an enzyme obtained from filtered autolyzates of cultures of a *saprophytic* bacillus (*314*).

In spite of the chemical similarity between the pneumococcus polysaccharides type III and type VIII, the latter containing D-glucose and D-glucuronic acid in a 7:2 ratio, and in spite of the immunological analogy between the two polysaccharides of producing cross reactions, the enzyme action is limited to the type III polysaccharide, a rather remarkable phenomenon of enzymatic specificity.

The role of this enzyme to protect against pneumococcal infection

(*315, 316*) consists apparently in the hydrolysis of the capsular polysaccharide material, with the effect that the decapsulated bacteria become exposed to the action of phagocytes.

XI. Mucopolysaccharases

In accordance with the generally accepted nomenclature whereby all those polysaccharides that contain aminosugars are called mucopolysaccharides, the enzymes that hydrolyze this group of natural polymers are termed mucopolysaccharases. According to the chemical nature of their substrates, they are to be subdivided into three classes: (*a*) enzymes that hydrolyze mucopolysaccharides not containing uronic acid (chitinase, lysozyme); (*b*) enzymes that hydrolyze mucopolysaccharides containing uronic acids, but no sulfate (hyaluronidase); (*c*) enzymes that hydrolyze mucopolysaccharides containing sulfate (chondroitin sulfatase, heparinase).

A. Chitinase

This enzyme, although classified with the mucopolysaccharases because of the chemical nature of its substrate, has many common traits with cellulases and other enzymes that hydrolyze insoluble structural polysaccharides from the cell wall, in particular its occurrence in association with those enzymes.

Karrer and Hofmann (*317*) described chitinase in the digestive tract of the Roman snail (*Helix pomatia*). The product of reaction is *N*-acetylglucosamine. Fractionation of the enzymes from the snail and from almonds (emulsin) yielded a chitinase which did not split chitobiose, i.e., which produced low molecular chitodextrins rather than *N*-acetylglucosamine as end products (*318–321*). These authors also found the presence of a chitobiase in the crude enzyme solutions that they were able to separate from the chitinase by chromatography.

The importance of the *N*-acetyl groups of the substrate on the enzymatic degradation was pointed out by Karrer (*253*). When partially deacetylated chitosan served as substrate for the enzyme, the end products of the reaction were tri- and tetrasaccharides. Only after acetylation of their amino groups were these dextrins again available for enzymatic attack.

Besides the occurrence of chitinase in the snail and in plants (emulsin), this enzyme has also been found in molds (*322*), bacteria, and actinomyces (*295*). Chitinase, as well as chitobiase, is secreted by the epidermis of arthropods (*322a,b*), by the intestinal walls of earthworms (*322c,d*), and by the hepatopancreas of crabs (*322e*). This enzyme has been purified from *Streptomyces* cultures (*322f*).

B. HYALURONIDASE

1. *Mechanism of Action*

There are considerable analogies between the actions of α-amylase and hyaluronidase. In both cases, the initial degradation produces a rapid decrease in the viscosity of the substrates, which, in the case of hyaluronidase, is accompanied also by a decrease in the capability of the substrate to be precipitated by proteins. Although the reducing power of the substrate increases only very slightly during this first stage of α-amylase and hyaluronidase actions, prolonged enzymatic digestion produces reducing sugars in both enzymatic reactions. The end products are oligosaccharides in both cases, the nature and degree of polymerization of which depend on the source of the enzyme. Thus, the products of exhaustive action of barley malt α-amylase on amylopectin are known to be of higher molecular weight than the end products of salivary α-amylase digestion (*39*). In analogy, testicular and snake venom hyaluronidases, splitting endohexosaminidic bonds of the substrate, yield mainly *tetrasaccharide*, with disaccharide appearing only as a minor component (*323*), whereas hyaluronidase from microorganisms, splitting the same bond, yields a $\Delta^{4,5}$ (glucuronide) unsaturated *disaccharide* (*324*), whereby the unsaturation was introduced during the enzymatic digestion. In addition, hyaluronidase of the leech (*Hirudo medicinalis*) is of a different type since it hydrolyzes endoglucuronidic bonds; it yields a tetrasaccharide like the testicular enzyme, with the exception, however, that the latter has a hexosamine at the reducing end group while the former has a uronic acid (*325*).

The action of hyaluronidase on capillary permeability was first reported by Duran-Reynals (*326*); the activity to increase capillary permeability is known as spreading factor and has been identified with the enzymatic activity of hyaluronidase (*327*). This physiological phenomenon has no precedents with other polysaccharidases.

2. *Occurrence*

The main source of *mammalian* hyaluronidase is found in testicles; bee and snake venoms (*328*) and leech heads (*329*) have also been reported to contain hyaluronidase activity. Reports on the presence of hyaluronidase in other mammalian tissues, such as spleen, ciliary body and iris (*330*), cornea and skin (*331*), have been attributed to bacterial contamination (*328, 332*). Salivary hyaluronidase (*65*) is decreased upon penicillin administration; it is probably a bacterial enzyme (*333*).

No hyaluronidase activity has been reported in higher plants, but the occurrence of this enzyme in bacteria is most frequent. Extracts of pneumococci of types I, II, III, VI, VIII, and XIII (*328, 334–336*), of *Clostridium welchii* (*330*), *Clostridium perfringens* (*334*), strains of hemolytic streptococci and staphylococci have been found to contain hyaluronidase (*332*).

3. *Properties*

The principal properties of testicular and bacterial hyaluronidases have been summarized by Meyer (*337*) and are presented in Table XXIV.

TABLE XXIV

PROPERTIES OF TESTICULAR AND BACTERIAL HYALURONIDASE[a]

Property	Testicular hyaluronidase	Bacterial hyaluronidase
Activity	Endo-β-hexosaminidase Transglycosidation	Endo-β-hexosaminidase No transglycosidation
Stability	Thermostable	Thermolabile
Substrate affinity	Increases with molecular weight	Nearly independent of molecular weight
End products	Mainly tetrasaccharide plus disaccharide as minor component	Disaccharide only
Disaccharide	β-D-glucosylpyruronic acid, 3-*O*-*N*-acetylglucosamine	β-D-glucos-4,5-enylpyruronic acid, 3-*O*-*N*-acetylglucosamine

[a] According to K. Meyer (*337*).

Leech hyaluronidase is distinct from both the testicular and bacterial enzymes by the different type of linkage it hydrolyzes. It resembles the testicular enzyme in that it produces mainly a tetrasaccharide, whereas its property of not catalyzing transglycosidation is more related to the bacterial enzyme.

The transglycosidation reaction catalyzed by testicular hyaluronidase was described by Weissman (*338*) and was found to produce an increase in the molecular weight of oligosaccharides by transferring disaccharide units from one substrate molecule to another. Such a reaction, although not unique for hydrolytic enzymes, has not been reported for other polysaccharidases.

4. *Conclusions*

Hyaluronidase, although rather similar in its mode of action to α-amylase, is different from most other polysaccharidases in that, in addition to its hydrolytic activity, it is capable of catalyzing a transglycosidation reaction; this has not been reported with any of the other polysaccharide hydrolyzing enzymes, but it is not infrequent in other hydrolytic enzymes.

Hyaluronidase is further distinct from the other polysaccharidases by its physiological action on the capillary permeability, a reaction unique not only among hydrolases.

C. HEPARINASE

A soil bacterium identified as *Flavobacterium* has been isolated in pure form, which is able to use heparin as its sole source of carbon, nitrogen, and sulfur (*339*). It is implied that this microorganism must contain an enzyme capable of depolymerizing heparin, although the mechanism of this reaction is still unknown. The presence of heparinase in tissue extracts, in particular from rabbit liver, has been reported by Jaques (*340*). This author measured heparinase activity by the disappearance of the anticoagulant activity of the substrate. The nature of the chemical reaction underlying this inactivation is still unknown; it does not necessarily have to be a depolymerization reaction, although this appears to be quite likely.

D. CHONDROITINASE

Two enzymes appear to be involved in the breakdown of chondroitin sulfate from cartilage. A successful separation of these two enzymes has been achieved by Dodgson and Lloyd (*341*). They observed that the hydrolysis of sulfate ester groups by chondrosulfatase is not a prerequisite for the depolymerization of the substrate by chondroitinase, but the activity of the sulfatase is greatly enhanced by preliminary degradation of the polysaccharide chain. This indicates that the depolymerization must be the primary reaction whereby the formation of sulfated oligosaccharides can be anticipated.

Chondroitinase has been found in *Proteus vulgaris* (*341*), and the presence of this enzyme in association with the sulfatase was also reported in other bacteria (*342*) and in mollusks (*343*).

The degradation of chondroitin sulfate by certain hyaluronidase preparations has been reported in the literature (*344*). It is not known, however, whether these enzyme sources contain a specific chondroitinase in addition to hyaluronidase, or whether the specificity of hyaluronidase in these preparations has a broad spectrum of substrate specificity which would also include chondroitin sulfate.

E. LYSOZYME

Lysozyme is the example of an enzyme which was discovered (*64*) through its physiological action (dissolution of certain bacterial cells).

purified, and obtained in the crystalline form (*345*) before the chemical nature of its substrate was known. The bacteriolytic action of lysozyme on certain microorganisms, in particular *Micrococcus lysodeikticus* and *Sarcina lutea* was for some time the only known activity of this enzyme.

In 1946, Meyer and Hahnel (*346*) fractionated the cell material of *Micrococcus lysodeikticus* and of certain other microorganisms and obtained a highly polymerized phosphomucopolysaccharide (*1*), containing 5.5–6.5% nitrogen and 23–30% hexosamine. This material was found to be a suitable substrate for lysozyme. The enzyme was able to reduce the high viscosity of the substrate solutions in an initial reaction and to produce reducing substances during the later stages.

Considerable similarities exist between the modes of action of lysozyme and hyaluronidase. Both enzymes catalyze the depolymerization of their respective substrates, first producing a marked decrease in the viscosity and later an increase in the reducing power. Both enzymes can be reversibly oxidized.

Lysozyme was originally found in nasal mucus and in egg white, from which latter source it has been obtained in the crystalline form. The occurrence of lysozyme in the latex of different plants, i.e., *Ficus* and *Papaya*, has been demonstrated, and the enzymes of plant and animal origin have been compared (*347*). *Ficus* lysozyme is different from the egg white enzyme in its pH optimum of action, its stability, electrophoretic behavior, ability to be adsorbed on bentonite, and behavior toward some strains of *Micrococcus lysodeikticus* and *Sarcina*. Lysozyme also occurs in microbes, in particular in those susceptible to the enzyme (*348*). The physiological role of lysozyme has been discussed by Meyer (*346*), who believes that its function appears to be primarily metabolic, concerned mainly with the depolymerization and breakdown of its substrate. In this case, as Meyer pointed out, one would have to anticipate a wide occurrence of its substrate which, so far, could not be observed. The role of lysozyme as a bacteriolytic agent, i.e., its role as a defense mechanism, might be incidental.

XII. General Discussion

The principal function of the large majority of polysaccharidases appears to consist in their ability to transform high molecular and, in many instances, totally insoluble, substrates into water-soluble, low molecular substances. Unlike polysaccharides, the products of polysaccharidase action are capable of penetrating into the cells where they may subsequently undergo further metabolic changes. Consequently, most of the polysaccharidases, whether they occur in animals, higher plants, or microorganisms, are extracellular enzymes. This is particularly true for the large group of enzymes capable of destroying the extremely insoluble structural materials from the cell walls of plant tissue, i.e., cellulases, lichenase, mannase, xylanase, hemicellulases, the pectic enzymes, etc.

Most polysaccharidases acting on soluble substrates are also of extracellular nature; they are secreted by various glands of the animal organism, such as α-amylase from the salivary glands and from the pancreas, or they are emitted into the culture media by bacteria and molds, like amylase from *Bacillus subtilis* and *Aspergillus oryzae* and like dextranase from *B. cellvibrio* and from various *Penicillium* strains.

Another group of extracellular polysaccharidases are the enzymes capable of dissolving the more or less gelatinous mucopolysaccharides surrounding the mammalian ovum and certain bacteria, e.g., hyaluronidase from the semen and from bacteria, and lysozyme.

Only a few polysaccharidases are known to be intracellular enzymes, such as the α-amylase occurring in rather low concentrations in various mammalian tissues. Their role is not quite clear, however, and it may well be that they fulfill an entirely different function from that of the extracellular pancreatic and salivary amylases. The possibility that liver amylase may be involved indirectly in the synthesis of glycogen by maintaining at a constant level the concentration of low molecular polysaccharidases. The enzymes acting on insoluble substrates form a action, has been discussed earlier in this chapter.

As it was pointed out before, there is a close relationship between the extracellular nature of these enzymes and their biological function. Many of the completely insoluble structural polysaccharides of plant cell walls, such as cellulose, lichenin, mannan, galactomannan, xylan, hemicellulose, some pectic substances and, in addition, chitin. may be made available as a source of energy to organisms excreting specific polysaccharide or oligosaccharide primers, necessary for phosphorylase distinct group not only because they have the ability in common to solubilize their substrates, but also because they closely resemble one another with respect to their distribution in nature. Thus, they are found in very similar places; i.e., bacteria and molds and, in a few instances, in higher plants (barley, potato sprouts), in protozoa (amebas), and in the intestinal tracts of mollusks and insects. Their occurrence in the animal sources, especially in insects, in clams and some other mollusks is due, at least in part, to the intestinal flora and fauna of the animals, rather than to animal origin itself. One comes to the conclusion, therefore, that the digestion of structural plant cell wall polysaccharides and of chitin is mostly, if not entirely, carried out by bacteria, molds, and protozoa, and that lower animals utilizing insoluble polysaccharides as food, have to rely for this purpose on their intestinal fauna and flora.

It can further be concluded from the above considerations that the distribution in nature of the polysaccharides is completely unrelated to the distribution of their specific hydrolytic enzymes. This is also particularly evident in the case of dextran, which is synthesized exclusively by, and which occurs solely in, certain bacteria, whereas dextranase is found in molds and even in mammalian tissues as well as in bacteria.

There is considerable consistency as to the general mechanism of action of polysaccharidases. In the case of substrates having more than one type of bond to be hydrolyzed, there exists a separate enzyme specific for each bond; i.e., the α-1,4-glucosidic bonds in amylopectin and glycogen are broken down by the amylases; the α-1,6-glucosidic bonds by more or less specific glucosidases; the glycosidic bonds of the pectic substances and their methyl ester linkages are hydrolyzed by polygalacturonase and by pectinesterase, respectively; hyaluronidase (of testicular and bacterial origin) splits only the hexosaminidic bonds of the substrate, whereas the glucuronidic bonds of the split products may be hydrolyzed by β-glucuronidase; chondroitin sulfate is depolymerized by chondroitinase; and chondrosulfatase hydrolyzes the sulfate ester bonds.

Where the mechanism has been studied in some detail, it appears that the type of linkage occurring in the majority is broken down first, like the α-1,4-glucosidic bonds in amylopectin and glycogen. In some cases, the depolymerization, i.e., the splitting of glycosidic bonds of the polymer chains, may precede the hydrolysis of the accessory bonds, i.e., of the bonds which do not contribute to the polymer nature of the substrate; e.g., during the degradation of chondroitin sulfate where the hydrolysis of the sulfate ester groups was found not to be an essential prerequisite for the depolymerization; or in the breakdown of chitin where the presence of *N*-acetyl groups was even necessary for the action of chitinase. On the other hand, a reversed situation appears to exist in the case of the pectic enzymes, in which the splitting of the methyl ester bond has to precede the action of polygalacturonase; the latter enzyme has been found, indeed, to hydrolyze only those glycosidic linkages that are not adjacent to esterified galacturonate units.

The actual depolymerization of polysaccharides by enzymes is in most, but not in all, cases achieved in a two-stage reaction. During the first step, the glycosidic bonds of the polymer are randomly split by a first enzyme (with the exception of β-amylase) until mainly disaccharides and a few other oligosaccharides of low degrees of polymerization, ranging from two to six, are formed. Then, another enzyme hydrolyzes the di- and oligosaccharides to the monomer. This mechanism has been found to prevail during the biological breakdown of amylose by α-amylases and maltases, of cellulose by cellulase and cellobiase, as well as in some less-known systems, as in the degradation of chitin by chitinase to chitobiose. An exception to the two-stage mechanism is the degradation of amylose by glucamylase from fungi, and it is believed that the one-stage mechanism of degradation may be preferred by some lower organisms.

In some instances, it has been suggested that the depolymerization of insoluble polysaccharides is carried out in a three-stage reaction. Such a mechanism is similar to the two-stage reaction discussed above, with the exception that the random depolymerization of the soluble polysaccharide is preceded by another stage during which the insoluble high molecular substrate is transferred into a soluble, but still high molecular, compound. The mechanism of such a reaction is probably identical with that of the depolymerization which follows, and it may be a matter of further research to decide whether the enzymes acting on the insoluble substrate are actually different from the ones that depolymerize the soluble polysaccharide. An example of the three-stage mechanism is the system of the C_1 and C_x cellulases, the former being capable of solubilizing cellulose to some extent, the latter being the actual cellulase which acts upon the high molecular, but more or less soluble, substrate. Another example is the protopectinase-depolymerase-polygalacturonase system.

A rather unique enzyme within the group of polysaccharidases is β-amylase. Its mechanism of action is entirely different from the type involving random splitting which has been discussed above and which appears to be rather general. β-Amylase is also peculiar in its almost exclusive occurrence in higher plants, but its role in nature is not well understood.

Polysaccharidases may include the first enzymes ever discovered and studied by men, but much is still to be learned about them.

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CHAPTER 9

Saccharides: Alternate Routes of Metabolism*†

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† The following abbreviations are used throughout this paper: ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide; E-4-P, erythrose-4-phosphate; F-6-P, fructose-6-phosphate; FMN, FAD, flavin mono- or dinucleotide; G-6-P, glucose-6-phosphate; GLA-P, glyceraldehyde-3-phosphate; HMP pathway, hexose monophosphate pathway (phosphogluconate cleavage; this usually does not imply the complete pentose cycle); KDPG, 2-keto-3-deoxy-6-phosphogluconate; 6-PGA, 6-phosphogluconate; PKPE, phosphoketopentose epimerase; PPI, pentose phosphate isomerase; R-5-P, ribose-5-phosphate; RNA, ribonucleic acid; RTU, relative time unit (time required for disappearance of an added test substrate from the medium); Ru-5-P, ribulose-5-phosphate; SH-7-P, sedoheptulose-7-phosphate; TPN, triphosphopyridine nucleotide; Xu-5-P, xylulose-5-phosphate.

The volume of literature dealing with the metabolism of carbohydrate-type materials has grown enormously in recent years, to the point that the authors have felt obliged to restrict the scope of this review within more narrow limits. It has been decided to adhere to a fairly literal interpretation of "saccharides" and to omit from this discussion extensive mention of the metabolism of partial oxidation products of saccharides, e.g., pyruvate. We will direct our attention to the metabolism (chiefly degradative) of saccharides per se, particularly of glucose and of a few of its close relatives. Partial oxidation products, when they are discussed at all, will be mentioned from the standpoint of saccharide metabolism, either as direct end products or as they may be situated on the mainstream of terminal oxidation routes that supply large amounts of energy from the oxidation of glucose.

In a previous volume, the traditional breakdown routes of carbohydrate metabolism, i.e., glycolysis and the Krebs citric acid cycle, have been discussed.* In the minds of many biochemists, the central position of these pathways is partly deserved, partly traditional. Other newer metabolic routes which have been studied in detail in more recent years have been shown, as will be described in these pages, to be of varying significance on a comparative basis, and even (as the pentose cycle) to be of as widespread occurrence as are the classic pathways referred to above.

I. The Pentose Cycle

A. HISTORICAL INTRODUCTION

This route of carbohydrate breakdown, otherwise called the hexose monophosphate "shunt," the "direct" oxidative pathway, the phosphogluconate oxidation scheme, or the pentose phosphate cycle, has been recognized *in toto* since 1953, when Horecker described its operations in considerable detail (*1*). Several stimulating reviews on the subject have appeared (*2, 2a, 2b, 2c, 3*). Important fragments of this cycle, however, have been recognized for nearly a quarter of a century, since Warburg *et al.* (*4*), Lipmann (*5*), and Dickens (*6*) discovered that glucose-6-phosphate could be oxidized to yield pentose without the intervention of glycolysis.

Indeed, in retrospect, the reader may wonder why these principal discoveries did not immediately trigger a more widespread search for the details of this oxidative scheme; the citric acid cycle was actually announced later (*7*) than the three papers cited in the foregoing paragraph. That these discoveries did not evoke more widespread interest at the time was probably due to at least three major factors. (*a*) Adequate methods for detecting the various sugar phosphates that are intermediates in the pentose cycle were not yet available, hence additional steps in the process went undetected. (*b*) Most of the components steps in the Krebs cycle were already known, so that when the Krebs cycle was described it could immediately be tested in a variety of ways. When this was done in many types of tissues during the 1940's, the enzymes of the Krebs cycle were found to be both widespread and abundant. The localizing of these enzymes in the mitochondria (*8*), and the description of the mitochondria by Claude as the "intracellular power plant" of the cell, placed the citric acid cycle in a position of prominence as a terminal oxidation mechanism. (*c*) The production of ribose phosphate by the *Zwischenferment* enzyme of Warburg seemed to provide a happy solution to the question of the origin of pentose for RNA synthesis. Despite fairly clear statements that a new scheme might exist for the oxidation of glucose which was different from glycolysis or the Krebs cycle (*9*, *10*) these suggestions appear to have had little influence at the time. Several people were working in the field during the period 1938–1951, but the fate of triose phosphate was clouded; it seemed possible that it could be removed as formed via oxidation by the Krebs cycle, as if in a "shunt" mechanism; indeed, the term "hexose monophosphate shunt" became a popular name for the oxidation of 6-phosphogluconate. It was only after the recognition that two moles of pentose phosphate combine to form a mole of sedoheptulose phosphate (*11*) and that the triose phosphate formed was not oxidized but was probably trapped as pentose, sedoheptulose, or hexose phosphate (*12*), that the complexities of the new mechanism became appreciated by large numbers of biochemists. The work of Horecker (*1*) and Racker (*2*) and their respective colleagues presented the pathway in its true identity, as an independent cycle, in which under certain circumstances glucose could be oxidized completely to carbon dioxide and water without reference to either glycolysis or the Krebs cycle. Others have contributed significant information that assisted in establishing the pentose cycle; these are discussed in the reviews referred to earlier (*2*, *3*). Still others have contributed to the general recognition of the importance of the pentose cycle in nature; some of these will be referred to in the pages that follow.

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* See Vol. I, Chapter 9.

B. OUTLINE OF THE PENTOSE CYCLE

Respiration via the pentose cycle is outlined in Fig. 1. For convenience, six molecules of glucose-6-phosphate (G-6-P) are taken to represent the total pool of sugar used and are represented as hexose phosphate, or C_6P. The diagram may be evaluated more easily by considering at first only its left half: three molecules of G-6-P are degraded, and two molecules of fructose-6-phosphate are regenerated together with a molecule of triose phosphate. By doubling the lot, the two triose phosphates can be shown to combine to form a molecule of hexose diphosphate; or put another way, the six original molecules disappear, to be replaced ultimately by five molecules of the same material. The missing carbon atoms are accounted for as CO_2, through the process of G-6-P and 6-phosphogluconate oxidation. These oxidations are the ones originally studied by Warburg, Lipmann, and Dickens and can be regarded as the "direct" oxidative portion of the cycle. The various sugar phosphate dismutations, which are described below, are nonoxidative; they result in reappearance of hexose phosphate, which can recycle to produce further (net) oxidation of hexose.

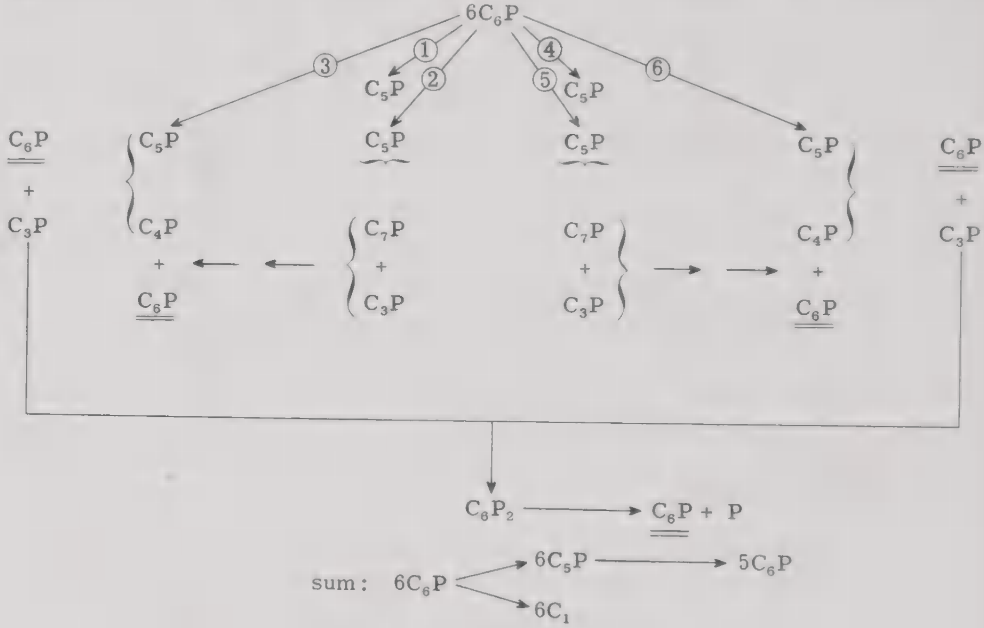

FIG. 1. The pentose cycle.

Figure 2 depicts the pentose cycle in a different fashion, including the fate of the individual carbon atoms of glucose as they traverse the respective steps. Two molecules of glucose are employed at the outset: one, in which the respective carbon atoms are labeled 123456, the other ABCDEF. The first two steps (Reaction 1) result in the loss of the aldehydic carbon, producing carbon dioxide and leaving two molecules of pentose phosphate that correspond to the bottom five carbons of hexose phosphate. The pentose phosphate next undergoes a transketolase reaction (Reaction 2) in which the top two carbons of xylulose phosphate become the top two carbons of sedoheptulose phosphate. This reaction is followed by a second dismutation (Reaction 3), catalyzed by transaldolase, in which the top three carbons of the heptose "return," so to speak, to add onto the triose moiety produced from Reaction 2; thus a molecule of hexose (fructose) phosphate is regenerated and tetrose phosphate is also formed. In an aerobic system, the top carbon of fructose (corresponding to carbon 2 of the original glucose) can now be lost by the *Zwischenferment* and 6-PGA[2] dehydrogenase reactions; thus in this scheme carbon 2 of glucose emerges as CO_2, slightly behind carbon 1 (= A), which was released as a product of Reaction 1.

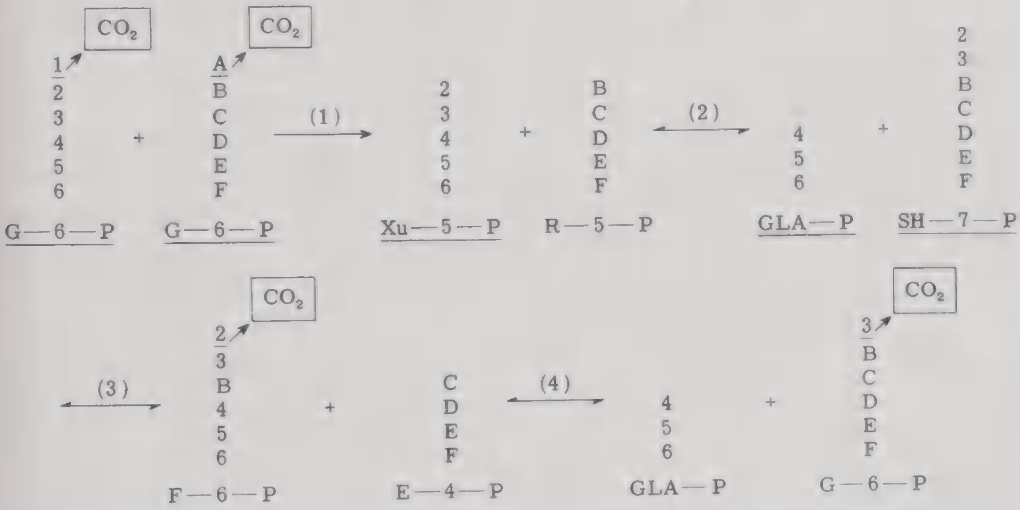

FIG. 2. The pentose cycle: fate of individual carbon atoms.

The pentose phosphate that arises from Reaction 3, can now rearrange with tetrose phosphate in a second aldolase reaction (Reaction 4); the products are a second molecule of fructose (with the first carbon corresponding to carbon 3 of the original glucose) plus a molecule of triose phosphate. However, when this first carbon atom of fructose phosphate is released by oxidation, the pentose that remains is labeled BCDEF—exactly as in the first pentose formed from Reaction 1. Thus, trace amounts of this species of pentose could theoretically catalyze the oxidation of all the remaining hexose phosphate. In actual practice the type BCDEF may be diluted, since as Horecker and coworkers (*13*) and Beevers (*14*) have pointed out, the tetrose phosphate (CDEF) formed by transaldolase in Reaction 3 may interact with "fresh" pentose, i.e., pentose phosphate newly formed from phosphogluconate decarboxylation. This is shown in Fig. 3. To the extent that this variation occurs, the hexose phosphate formed in Reaction 4 will show inversion of the first two carbons, with the sequence 23CDEF (or BCCDEF), as shown in Fig. 3. Oxidation of such a species would enrich the respiratory CO_2 with carbon 2, and the appearance of carbon 3 would be slightly delayed, even beyond the normal sequence to be expected from the pentose cycle.

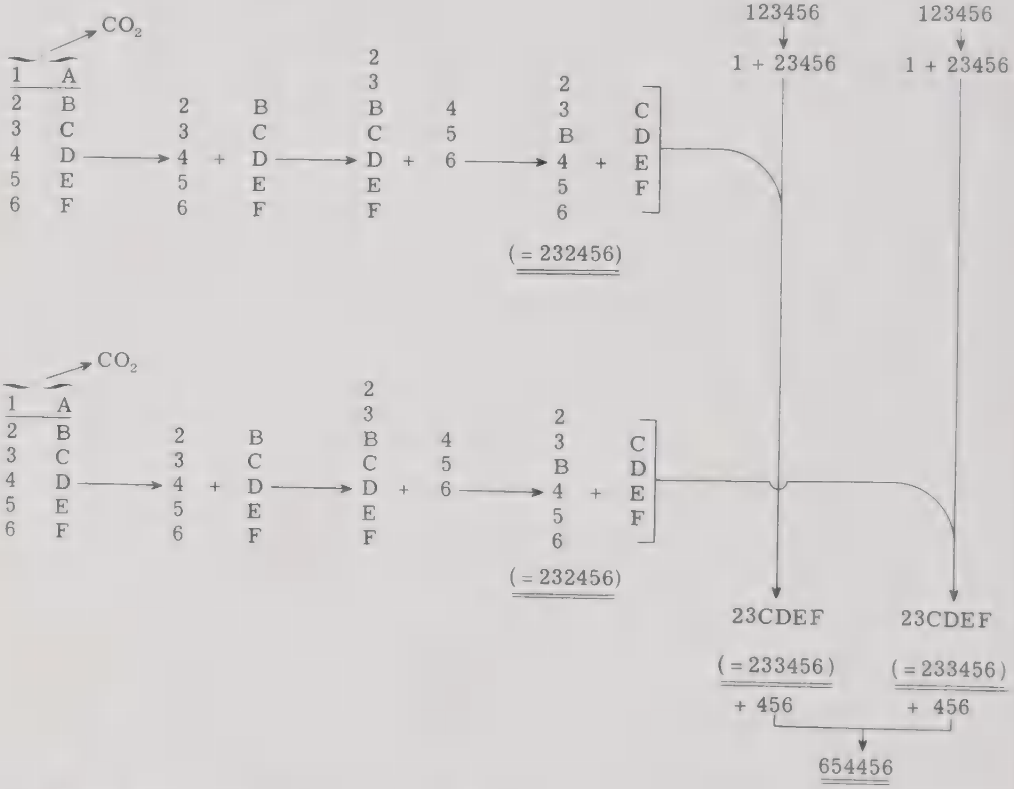

FIG. 3. Outline of the pentose cycle. A modified version, similar to that depicted by Beevers (*14*).

Carbons 6, 5, and 4, according to the pentose cycle, can appear in CO_2 only after recombination of triose units. This is really a scheme for direct oxidation of the first carbon atom of glucose, with carbons 2 and 3 assuming the top, or oxidizable, position after rearrangement of the sugar phosphates. The hexose that is formed from recombined triose phosphate will have the sequence 654456 of the original glucose. It will lose carbon 6, followed by carbons 5 and 4, but (until the oxidation of glucose is complete) always at a retarded rate and in reduced amount because of retention of the bottom 3 carbons of every hexose molecule that enters the cycle.

When the individual glucose atoms are identified by specific labeling with C^{14}, the foregoing statements can be subjected to experimental verification (*15*). The data that can be obtained from appropriate experiments can be employed to investigate the comparative aspects of oxidative pathway participation, as will be seen in Section V, Comparative Studies.

C. ENZYMES OF THE PENTOSE CYCLE

The enzymes of the pentose cycle have been investigated in detail, both per se and with regard to the formation of products. They are listed below.

1. *Hexokinase*

This enzyme has been studied in many laboratories. Because it has usually been assumed to be the primary step in glycolysis, hexokinase activity has in the past been taken to indicate the presence of the latter pathway. However, in view of the reactions outlined in Fig. 2, it is clear that the presence in an organism of hexokinase may be just as indicative of the formation of glucose-6-phosphate for pentose cycle oxidations as for glycolysis.

A review of hexokinase and of other 1- and 6-kinases of hexoses has been presented (*2*).

2. *Glucose-6-phosphate Dehydrogenase*

This enzyme is one of the most ubiquitous that has been found in nature. It has been described as occurring, for example, in animal tissues (*16–34*), worms (*35, 36*), marine forms (*37, 38*), insects (*39–44*), higher plants (*45–51*), bacteria (*52–70*), and fungi, including yeast (*71–86*). Of these, however, until recently the enzyme has been only partially purified (especially from mammalian sources) and the major source of relatively pure G-6-P dehydrogenase has been from yeast. The yeast enzyme has been purified 600–900-fold by means of fractionation with ammonium sulfate, adsorption on calcium phosphate gel, and chromatography on starch-Celite columns.*

Some pertinent points about the yeast enzyme are given in Table I, and a comparison is made with the enzyme from some other sources. It may be seen that G-6-P dehydrogenase is present in different types of tissue in quite different amounts; to produce the purified yeast

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* Very recently Julian, Wolfe, and Reithel (*23*) isolated G-6-P dehydrogenase in crystalline form from mammary gland (see Table I).

TABLE I

COMPARISON OF CHARACTERISTICS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE FROM DIFFERENT SOURCES

Source	Substrate	Coenzyme specificity	Product	Fold purification	Separation of G-6-P, 6-PGA dehydrogenase	Highest specific activity[a]	Optimum pH	Substrate K_m	References
Yeast	D-Glucose-6-PO_4	TPN, Mg or Mn	D-α-Gluconalactone-6-PO_4	600–900	Complete	86	8.5	$2.0–6.9 \times 10^{-5}$	*79–83*
Escherichia coli	D-Glucose-6-PO_4	TPN, Mg	D-α-Gluconalactone-6-PO_4	11	2% 6-PGA dehyd.	7	7.7–8.6	3×10^{-4}	*52*
Leuconostoc sp.	D-Glucose-6-PO_4	TPN, DPN	D-α-Gluconalactone-6-PO_4	12	Not stated	39	7.8	5.3×10^{-4}	*56, 57*
Acetobacter suboxydans	D-Glucose-6-PO_4	TPN, DPN	D-α-Gluconalactone-6-PO_4	100	Complete	100	8.0	3.9×10^{-4} (DPN)	*66*
Neurospora crassa	D-Glucose-6-PO_4	TPN	D-α-Gluconalactone-6-PO_4	—	Complete	36	—	—	*69*
Aspergillus niger	D-Glucose-6-PO_4	TPN	D-α-Gluconalactone-6-PO_4	60	Complete	20	8.1–8.6	1.7×10^{-4}	*75*
Azotobacter vinelandii	D-Glucose-6-PO_4	TPN, DPN	D-α-Gluconalactone-6-PO_4	25	—	11	—	—	*53*
Liver									
Rat	—	TPN	—	—	—	—	—	5×10^{-5}	*20*
Horse	—	TPN	—	—	—	—	—	—	*20*
Kidney (rat)	—	TPN	—	—	—	—	—	—	*20*
Mammary tissue	—	TPN	—	10,000	Complete (crystalline)	420	—	—	*23*

[a] One unit = amount causing 1.0 optical density change at 340 mμ, under the conditions listed in the references. Specific activity = units per minute per milligram protein.

enzyme (specific activity = 86) required several hundredfold concentration. The bacterial enzyme employs either pyridine nucleotide as cofactor, whereas that from mammalian or plant sources tends to be specific for TPN+. The isoelectric point for the enzyme from frog muscle (*17*) is reported to be at pH 4.8, whereas that from rat blood is 5.85 (*17a*). Bivalent ions have been shown to activate the enzyme from yeast and *Escherichia coli*, but this has not been observed with G-6-P dehydrogenase from other sources.

3. *6-Phosphogluconate Dehydrogenase*

This enzyme, like glucose-6-phosphate dehydrogenase, has been reported active in many of the systems referred to in the preceding section; this is to be expected since 6-phosphogluconate is the product of G-6-P oxidation.

6-PGA dehydrogenase is further similar to G-6-P dehydrogenase in that its purification has been emphasized from microbial systems. Extensive purification of 6-PGA dehydrogenase has been carried out using yeast (*86*), *Leuconostoc* (*57*), *E. coli* (*52*), and *Acetobacter suboxydans* (*66*). Plant materials, although mentioned as a source of this enzyme (*25, 51, 87*), have not been extensively exploited. The enzyme from *A. suboxydans* has been purified most by means of electrophoresis on a starch column, so that it is free from G-6-P dehydrogenase (*66, 88*). Table II contains some comparisons of the enzyme from different sources.

The oxidation of 6-PGA by TPN involves both a decarboxylation and a dehydrogenation. As DeLey and Verhofstede (*89, 90*) have pointed out, the explanation for the mechanism of this reaction has shifted back and forth between the speculation that either 2-keto- or 3-keto-6-PGA was formed or that 2, 3-enediol gluconolactone-6-phosphate might also be an intermediate. The idea that 2-keto-6PGA served as the intermediate was attractive also to Uehara and Akabori (*91, 92*), who suggested that 2-keto-6-PGA might undergo an aldolase cleavage to hydroxypyruvate and glyceraldehyde-3-phosphate. This suggests a reaction similar to the cleavage of 2-keto-3-deoxy-6-phosphogluconate to yield pyruvate and glyceraldehyde-3-phosphate. (The latter reaction, found in pseudomonads, is a key cleavage in the Entner-Doudoroff pathway, discussed in Section IV.) Against this idea is the observation (*90*) that the proposed lactone of the 2,3-enediol is not metabolized in *Aerobacter* and that pentose phosphate enediol is apparently an artifact of an alkaline environment upon ribulose-5-phosphate. Moreover, 2-keto-gluconic acid-6-phosphate, when administered to *Aerobacter* (*89*) is

TABLE II

COMPARISON OF CHARACTERISTICS OF 6-PHOSPHOGLUCONATE DEHYDROGENASE FROM DIFFERENT SOURCES

Source	Coenzyme specificity	Fold purification	Separation of 6-PGA, G-6-P dehydrogenases	Highest specific activity[a]	Optimum pH	Substrate K_m	References
Yeast	TPN	45	30% G-6-P dehyd.	15.6	7.4	5×10^{-5}	*86*
Leuconostoc sp.	DPN	16	—	2.0	7.8	7.8×10^{-5}	*57*
Escherichia coli	TPN	—	4% G-6-P dehyd.	2.7	6.6–7.7	3×10^{-5}	*52*
Acetobacter suboxydans	DPN, TPN	—	Complete	50	8.0	2.3×10^{-4} (DPN) 1.3×10^{-3} (TPN)	*66*

[a] One unit = amount causing 1.0 optical density change at 340 mμ under the conditions specified. Specific activity = units per minute per milligram protein.

reduced to 6-PGA rather than oxidized to the expected product, ribulose-5-phosphate.

The alternate proposal, that 3-ketogluconate-6-phosphate may be formed as an intermediate in the oxidation of 6-PGA, was favored by Horecker and colleagues (*93, 94*) and by Racker (*2*), partly on the basis of the analogy with isocitric and malic dehydrogenases. This theory suffers from the uncertainty consequent upon the fact that the compound has not been tested because it is not available. Until this can be done, the proposed mechanisms must await verification. Meanwhile, it may seem reasonable to speculate that either or both 2-keto and 3-ketogluconate phosphates may function, depending upon the system and conditions used; either of the products (R-5-P or Ru-5-P) can be metabolized further by a suitable isomerase (see next section).

4. *Pentose Phosphate Isomerase; Phosphoketopentose Epimerase*

Reactions 1 and 2 of Fig. 2 are actually more complicated than is shown (*95, 96*); they should probably be represented as shown in Fig. 4.

In these reactions an isomerization occurs between R-5-P and Ru-5-P [enzyme:pentose phosphate isomerase (PPI)], and there is also an epimerization of the keto sugars Ru-5-P and Xu-5-P [enzyme:phosphoketopentose epimerase (PKPE)]. The subsequent reaction involving transketolase has been shown (*97, 98*) to act on Ru-5-P only after its conversion into Xu-5-P. The latter enzyme was at first called a Waldenase (*99*), but on the basis of evidence for a 3-ketopentose phosphate occurring as a minor product of the epimerization (*95, 100*) the name epimerase seems to be preferred. Although the mechanism of the epimerase reaction has not been determined, the formation of this 3-ketopentose phosphate as one of the products suggests the formation (*98*) of a 2,3-enediol phosphate as intermediate, and revives (by analogy) the prospect of 3-ketogluconate serving as intermediate in 6-PGA oxidation, as discussed in the preceding section.

Both the foregoing enzymes have been observed in animal tissues, bacteria, and plants. The mode of action appears to be the same from all sources. The aldo-ketopentose phosphate isomerase (PPI) enzyme was discovered by Horecker *et al.* (*94*) in yeast. The reported equilibrium ratio varies from 0.3 for Ru-5-P:R-5-P in yeast (*94*) to 0.5 in red cells (*2a*). In the PKPE reaction (*101*), the Xu-5-P:Ru-5-P ratio at equilibrium was 1.5. Dische and Shigeura (*102*), using calculations upon the enzyme from red cells and alfalfa (*103*), suggest that the various phosphates may coexist in equilibrium in the approximate proportions of 35% R-5-P, 16.5% Ru-5-P, 45.5% Xu-5-P, and 3% 3-ketopentose phosphate plus other analogs of pentose phosphate.* It is clear that, although the calculations differ slightly in the different systems cited, there exists a ready equilibrium which not only may permit an interchange of added hexose into xylulose phosphate, but also can admit pentoses into the scheme, as in *Lactobacillus pentosus* (*105*). Together with the activities of transketolase and transaldolase enzymes (see below), this

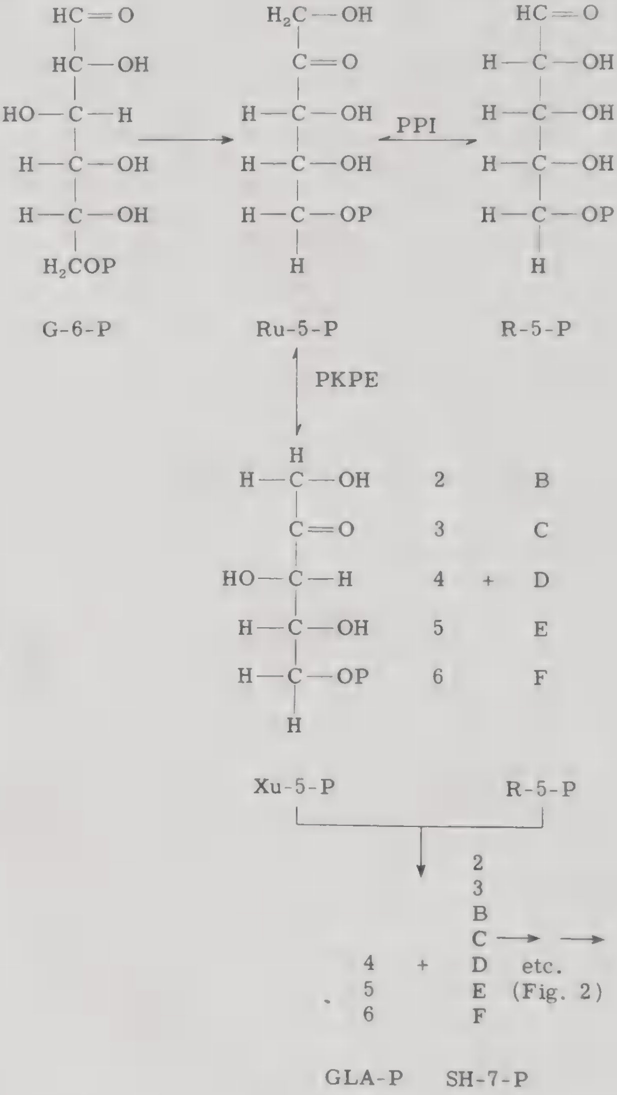

FIG. 4. The pentose phosphate isomerase (PPI) and phosphoketopentose epimerase (PKPE) reactions.

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* Tabachnik *et al.*, working with spinach and rabbit tissue enzymes, have calculated 47.7% R-5-P, 14.6% Ru-5-P, and 43.8% Xu-5-P (*104*) at equilibrium.

ease of interconversion readily explains the presence of so many isomeric hexoses and pentoses throughout nature.

PPI and PKPE have been purified extensively. PPI has been obtained from alfalfa (*106*) after a 350-fold purification that involved heating, fractionation with $(NH_4)_2SO_4$, and adsorption and elution from Celite. The enzyme appeared specific in its action on R-5-P and Ru-5-P. The pH optimum occurred at 7.0, and the turnover number was 240,000 moles per minute per 100,000 gm. of enzyme. PKPE has been purified 700-fold from extracts of *L. pentosus* (*101*) by $MnCl_2$ and $(NH_4)_2SO_4$ precipitation, adsorption and elution on calcium phosphate gel, and heating. The K_m for Xu-5-P was $5 \times 10^{-4}\ M$, whereas that for Ru-5-P was somewhat greater ($1 \times 10^{-3}\ M$).† As with PPI, the specificity of PKPE appeared to be restricted to its normal substrates. Neither enzyme has been found to require a dissociable coenzyme (e.g., uridine phosphate).

5. *Transketolase*

This enzyme has been purified from liver, spinach, and yeast (*107*, *108*), the greatest purification effort being applied to yeast. As a result of fractionation with acetone, alcohol, protamine, and ammonium sulfate, a crystalline preparation has been obtained that is reportedly 1200 times as potent as the starting material and has a specific activity of 150 units per milligram (*108*).‡ The best preparations from liver and spinach have reported activities of 3.0 and 29 units per milligram, respectively. No kinetic constants or other physical properties have been reported for the enzyme; however, it is known that thiamine pyrophosphate and Mg^{2+} are required for activity of transketolase. The equilibrium constant is about unity for the reaction. F-6-P + GLA-3-P → Xu-5-P + E-4-P (*109*).

The enzyme has broad specificity requirements, as shown in Table III. The "donors" are similar (except hydroxypyruvate) in that their first two carbon atoms resemble a glycolaldehyde unit, and the third carbon (the first asymmetric carbon) possesses an L configuration. The finding that Xu-5-P was active, not Ru-5-P, as a "donor" compound, helped to make clear this configurational requirement. Acceptor aldehydes, on the other hand, show less specificity; most are phosphorylated, but some, such as glycolaldehyde and glyceraldehyde, are not.

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† Tabachnick *et al.* (*104*) have purified PPI 150-fold from spinach leaves and PKPE 100-fold from rabbit muscle.

‡ One unit is the amount causing a change of 1.0 optical density at 340 mμ, resulting from oxidation of added DPNH by glyceraldehyde-3-PO_4 (a product of the reaction). Specific activity = units per minute per milligram protein.

TABLE III

SPECIFICITY OF TRANSKETOLASE

Donors of "active" glycolaldehyde ($CH_2OH—CHO$)	Reference	Acceptor aldehydes	Reference
Xylulose-5-PO_4	*97, 98*	Ribose-5-PO_4	*12*
Sedoheptulose-7-PO_4	*107*	Glycolaldehyde	*12*
Erythrulose	*107*	Glyceraldehyde-3-PO_4	*12*
Hydroxypyruvate	*91, 107, 108*	Glyceraldehyde	*12*
Fructose-6-PO_4	*110*	Deoxyribose-5-PO_4	*8*
Octulose-8-PO_4	*111*	Erythrose-4-PO_4	*111*

6. *Transaldolase*

This enzyme, which catalyzes Reaction 3 of Fig. 2, is reported to be specific for the indicated substrates. It has been concentrated over 400-fold (*112*) from brewers' yeast by fractionation with acetone, calcium phosphate gel, ammonium sulfate, acid, and ethanol. The reaction is freely reversible, with the equilibrium constant about 0.8.

II. The Glucuronate Pathway

There is increasing evidence that the metabolism of glucuronate may be facilitated by the pentose cycle. The reaction scheme envisioned by Eisenberg *et al.* (*113*) (Fig. 5) is as follows: D-glucuronate → L-gulonate (inversion of the carbon chain) → L-xylulose → xylitol → D-xylulose (inversion of the carbon chain) → D-xylulose-PO_4 → pentose cycle → glucose. Isotopic evidence (*113*) indicates that this may be a significant route for the metabolism of glucuronate in rat or guinea pig liver. On the other hand, the extent to which glucose may be converted to glucuronate is problematical. The evaluation of pathways, discussed in Section V will therefore neglect the possible formation of glucuronate from glucose; the participation of the pentose cycle would be measured regardless of the extent of this diversion of glucose carbon.

III. Heterofermentative Lactic Acid Degradation of Sugars

Certain heterofermentative lactic bacteria are able to degrade glucose by a series of cleavage reactions, as shown in Fig. 6.

This scheme, which appears to prevail in several species of *Leuconostoc* (*113a*), also serves to degrade pentose: The products are analogous with respect to the participating carbon atoms except (with pentose) that there is no carbon corresponding to glucose (C-1), hence no CO_2 is produced.

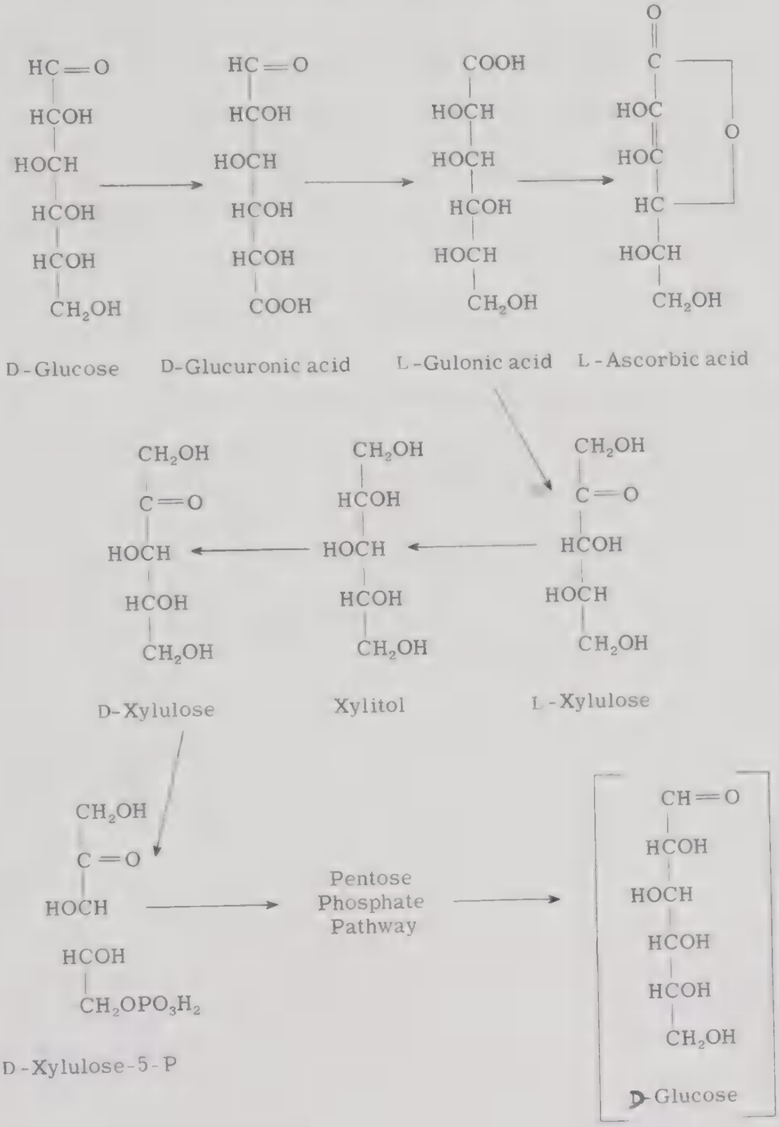


FIG. 5. Glucuronic acid pathway of glucose metabolism. After Eisenberg *et al.* (*113*).

In Section V phosphogluconate cleavage is measured by the appearance of C-1 of glucose in respiratory CO_2, in excess of the yield from C-6. CO_2 is also evolved in this heterolactic fermentation and, as in phosphogluconate cleavage, carbon 6 is not recovered in respiratory CO_2. However, the processes differ in that other carbon atoms of glucose are not concerned in CO_2 formation in the heterolactic fermentation. Carbon atoms 2 and 3 form ethanol or acetic acid; transketolase appears not to participate.

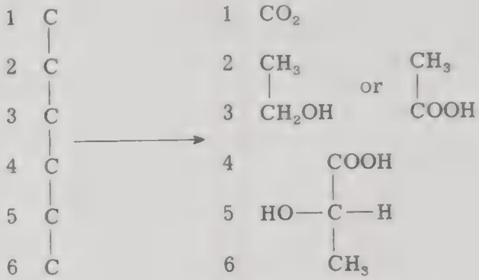
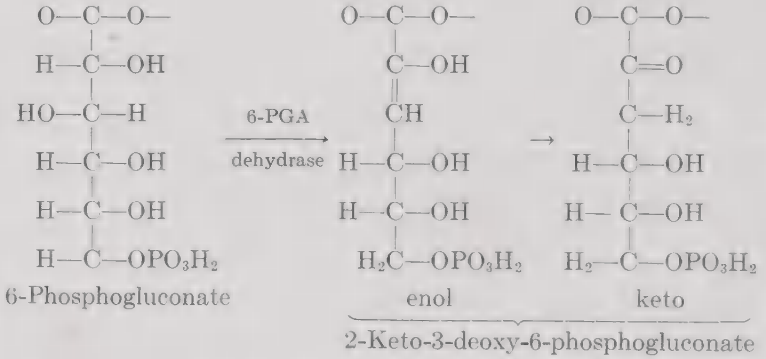


FIG. 6. The heterolactic fermentation.

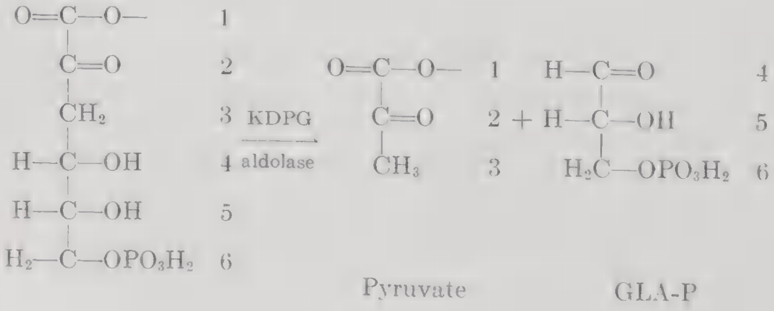
IV. The Entner-Doudoroff Scheme

This pathway of carbohydrate oxidation involves several of the reactions common to the pentose cycle, as well as to glycolysis (*114*). Thus, hexokinase and G-6-P dehydrogenase act sequentially on glucose, and the 6-PGA produced appears to be further metabolized to a compound which has been proposed to be 2-keto-3-deoxy-6-phosphogluconic acid (*115*). The enzyme, 6-PGA dehydrase, has been studied in *Pseudomonas fluorescens* and purified 27-fold (*115*). The reaction is pictured in Scheme 1:



(1)

The next enzyme is a key catalyst in this scheme; it is an aldolase called 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase, which cleaves the 6-carbon chain as shown in Scheme 2 (*115*):



(2)

The enzyme has been purified 25-fold from *P. fluorescens* (*116*). The products, pyruvate and triose phosphate, may then be utilized either by glycolysis or the pentose cycle. An important difference between this scheme and the two others just named lies in the source of the carbon atoms: pyruvate carbons 1, 2, and 3 correspond to carbons 1, 2, and 3 of glucose in that order, whereas from glycolysis the sequence is reversed. The order of the carbon atoms in triose phosphate is the same as in glycolysis. This difference permits analysis, by labeled C^{14}-glucose, of the routes of carbohydrate breakdown in an organism (*15*).

The enzymes of the Entner-Doudoroff pathway have been sought in a variety of species, but to date they appear to be restricted to a few pseudomonads (*114, 117*), *Azotobacter* (*118*), *Xanthomonas, Agrobacterium, Rhizobium* (*119*), and *E. coli* (*120*). Function of this route has not been demonstrated either in mammals or in higher plants, and it may be presumed, in view of the close relationships of the metabolic processes and the ubiquity of both glycolysis and the pentose cycle enzymes in many species, that 6-PGA dehydrase and/or KDPG may be absent in most organisms, despite the postulated existence of 2- or 3-keto-6-PGA in other species (*91–94*).

V. Comparative Studies

A. EXAMINATION OF METHODOLOGY

The recognition of alternate pathways for carbohydrate catabolism, particularly those for the hexoses and the pentoses, inspired students in biochemistry to estimate the relative participation of concurrent routes and to elucidate the specific roles played by individual pathways in cellular biosynthesis and respiration. Much has been accomplished recently in this area owing, at least in part, to the development of better research methods, such as radioactive tracer techniques and improved procedures for enzymological studies.

It is desirable first to discuss briefly the methodology involved in the study of pathway participation in both intact and cell-free biological systems. Here one finds that most of the reported methods involve two different approaches: (*a*) the detection of individual pathways, and (*b*) the estimation of pathway participation, analogous to the qualitative and quantitative analysis of a given problem. In the former, demonstration of the presence of individual enzymes associated with a pathway in a given biological system often is taken as presumptive evidence of the operation of the pathway in question. Details in this regard have been given in the preceding section. However, it should be stressed that the occurrence of a given pathway represents the sequential operation of a set of enzymatic reactions; confusion often resulted from findings in enzymological studies due to the many enzymes common to several catabolic pathways. Similar difficulties are also encountered in the quantitative evaluation of pathway participation inasmuch as the relative rates and extents of concurrent pathways often necessitates detailed kinetic information on the over-all catabolism of the substrate in question (*121*).

Radiotracer methods used for quantitative studies of catabolic pathways for carbohydrates can be generally classified into three major categories:

(1) Methods based on comparison of the specific activities of respiratory $C^{14}O_2$ derived from biological systems catabolizing C^{14} specifically labeled carbohydrates.

(2) Methods based on comparison of the specific activities of C^{14}-labeled metabolic intermediates derived from C^{14} specifically labeled substrates in a given biological system.

(3) The radiorespirometric method (based on the total *yield* of $C^{14}O_2$ from C^{14} specifically labeled substrates).

1. *Methods Based on the Comparison of the Specific Activities of $C^{14}O_2$ Derived from the Labeled Carbon Atoms of the Substrate*

Methods under this category were employed primarily to examine pathway occurrence and participation in mammalian tissue slices and plant tissue preparations (*14, 15, 122–128a*). Evidence collected in enzymological and other studies has indicated that glycolysis and phosphogluconate cleavage constitute the major mechanisms for hexose dissimilation, and consequently pathway estimations are made on this basis. Thus, the operation of the Entner-Doudoroff pathway, detected in microorganisms (*114*), is ignored since this route is yet to be demonstrated in either animal or plant tissues. It should be pointed out that inasmuch as a preparation of excised tissue does not generally utilize all or even a major fraction of substrate glucose in an incubation experiment, it is therefore necessary to use information on the specific activity of respiratory $C^{14}O_2$ from C^{14} specifically labeled substrates for pathway elucidations.

Qualitatively, the observation of a nonequivalence of the metabolic behavior of C-1 and C-6 of a hexose provides strong evidence for the operation of phosphogluconate cleavage in the system, since these two carbon atoms are converted similarly to the methyl carbon atom of pyruvate in the classic Embden-Meyerhof-Parnas pathway. It is assumed that there exists no preferential dilution of any labeled carbon atoms of the substrate from endogenous carbon sources. Consequently, the demonstration of a $C_6:C_1$ ratio, with respect to $C^{14}O_2$ production from glucose-6-C^{14} and glucose-1-C^{14}, respectively, of less than unity ($C_1:C_6$ greater than unity) has been used by many authors to indicate the operation of the HMP pathway in a given biological system (*51*, *129*). However, to estimate quantitatively the participation of each route, the use of data on the specific activity of $C^{14}O_2$ is subject to several serious problems. First, as pointed out by Wood (*121*) the dilution of individual carbon atoms of substrates by endogenous materials may depend on the pathways followed by the carbon atoms in question. Several workers (*122*, *124*, *125*) have attempted, with the aid of necessary assumptions, to determine the fate of the degradation products of hexose by the use of C^{14} specifically labeled intermediates known to be derived from glucose in glycolysis or the pentose pathways, as supplementary test substrates. Some notable examples are the use of C^{14} specifically labeled lactate and specifically labeled gluconate (*122*, *125*). By the use of specific activity data on $C^{14}O_2$ from glucose-1-C^{14}, glucose-6-C^{14}, or glucose-U-C^{14} together with that from the supporting substrates such as lactate, it was possible for these authors to devise equations for the estimation of pathway participations with respect to the origin of the respiratory CO_2. These equations depend upon the validity of the original assumptions, several of which need experimental verification. Also, the nature of this type of experiment is such that the data on the specific activity of $C^{14}O_2$ represent the *cumulative* information over a limited period of experimental time, subject to the deterioration of the excised tissue. Moreover, pathway participation calculated by these equations refers only to the fraction of respiratory CO_2 derived from an individual pathway, a piece of information not to be confused with pathway participation *in the total catabolism of glucose* inasmuch as there exists no ready correlation with regard to the amount of CO_2 produced via an individual pathway. It is obvious that the participation of pathways on the basis of total glucose catabolized is more meaningful. Equations have been devised by several authors (*126*, *127*) to estimate pathway participations in animal tissue making use of the data on the specific activities or yields of $C^{14}O_2$ from C^{14} specifically labeled glucose, lactate, and gluconate and on the amount of radioactivity incorporated into fatty acids from the respective labeled substrates.

More serious criticism of the validity of methods of this type was recently presented by Wood and Katz (*130*, *131*). These authors pointed out the necessity of taking into consideration the dilution of the administered labeled glucose by the glucose regenerated in the operation of the pentose cycle reactions. The dilution process is complex in nature, since the labeling pattern of the regenerated hexose phosphates is far different from that in the administered hexose. The administered hexose is therefore subject to drastic changes in specific activity of its carbon atoms, particularly in carbons 1, 2, and 3. In order to evaluate the extent of dilution of these carbon atoms, the authors carried out comprehensive calculations to account for the effect of recycling via the pentose cycle on the specific activity of glucose-6-phosphate derived from glucose-1-, -2-, and -3-C^{14}, respectively.

It is noted, however, that these calculations are based on the assumptions that fructose-6-phosphate derived from the pentose cycle operation is in perfect isotopic equilibrium with glucose-6-phosphate, derived from the administered labeled glucose, and that there exists no drainage of pentose cycle intermediates for other cellular functions, such as the use of pentose for nucleotide and nucleoside synthesis. Both these assumptions, especially the latter, represent limiting situations that may not be realized *in vivo.* Although knowledge is incomplete on the equilibrium: glucose-6-phosphate $\rightleftharpoons$ fructose-6-phosphate $\rightleftharpoons$ fructose-1,6-diphosphate in animal tissue, there exists evidence that in microorganisms, the fructose-6-phosphate derived from the pentose cycle can be readily converted to fructose-1,6-diphosphate, and hence trioses, via glycolysis. The portion of fructose-6-phosphate so catabolized will then interrupt the operation of the pentose cycle in the strict cyclic sense, making it impossible to calculate the exact dilution effect of regenerated fructose-6-phosphate upon the substrate glucose. The detailed experiments bearing on this point will be discussed later. As to the drainage of the pentose cycle intermediates into biosynthetic pathways, one may rationalize that it may not be important in isolated tissues, but it is certain that such processes do occur extensively in living systems.

2. *Methods Based on the Comparison of the Specific Activity of Metabolic Intermediates or Products Derived from C^{14} Specifically Labeled Substrates*

Methods of this type have been employed by many workers since the late 1940's. Thus, the Entner-Doudoroff pathway in *Pseudomonas saccharophila* was derived from the observation that C-1 of glucose or gluconate was converted to C-1 of the immediate catabolic product, pyruvate (*114*). However, it was 1954 before a workable method was developed by Blumenthal, Lewis, and Weinhouse (*132–134*) to correlate the specific activity of glucose-1-C^{14} or glucose-6-C^{14} to that of acetate, or some other metabolic product, for the estimation of pathway participation in yeast, *Pseudomonas fluorescens*, and liver tissue. The successful application of this method relies on the following characteristics:

C-1 of glucose is transformed into C-2 of acetate via glycolysis but is not incorporated into acetate at all by way of the pentose pathway, whereas C-6 of glucose can be transformed to C-2 of acetate via either route; the acetate or other intermediate can be isolated in sizable quantity. This is easily done in microbial or plant metabolism but is more difficult in many animal experiments, particularly when tissue preparations are used, since accumulation of intermediates is generally not realized. This difficulty is sometimes circumvented by the addition of unlabeled acetate or other intermediates to the incubation system to serve as trapping agent and to obtain a sufficient amount of labeled intermediate for analysis; however, the imposition of a nonphysiological condition to the experiment may upset the normal metabolism of the system under study.

The problem of the reduction of the specific activity of the metabolic product by nonlabeled diluent derived from endogenous material is another serious limitation of this method. However, the problem is handled ingeniously (*132*) through the use of glucose-U-C^{14} as sole substrate in a concurrent experiment. The specific activity of the metabolic product derived from glucose-U-C^{14} is then taken as the standard for the necessary correction for endogenous dilution.

It should be emphasized that the method as devised by Blumenthal, Lewis and Weinhouse (*132*) aims at the estimation of pathway participation with respect to the glucose administered. No consideration has been given to the dilution effect of regenerated fructose-6-phosphate upon the substrate glucose. Thus one of the conditions set forth by the authors requires that the pentose phosphate formed in the HMP pathway shall not cause the regeneration of fructose-6-phosphate. The extent of this dilution, as explained later in the text, is not likely to be great enough to affect the validity of the findings in an experiment using glucose-1-C^{14}, particularly if the operation of the pentose pathway is relatively minor in the over-all glucose catabolism.

More recently, the dilution of the administered labeled glucose by the hexose phosphates regenerated via the pentose cycle has been examined more closely by Dawes and Holms (*135–137*) in *Sarcina lutea*. These authors compared the fraction of conversion of the initial substrate, glucose-1-C^{14}, glucose-2-C^{14}, or glucose-6-C^{14} to the metabolic product pyruvate as an indicator of glucose catabolism in the organism. The dilution of the labeled glucose by regenerated hexose phosphates was considered by assuming that the fructose-6-phosphate produced via the pentose cycle could be catabolized further by both glycolysis and the pentose cycle, in the same proportion as could the substrate glucose. Equations were devised to calculate the theoretical radiochemical yields of pyruvate from the specifically labeled glucose substrates for a given extent of pentose cycle participation.

From the operation of the pentose cycle in Fig. 3, it can be readily visualized that the interfering effect of regenerated fructose-6-phosphate should be more pronounced when glucose-2-C^{14} is used as the tracing substrate and essentially insignificant when glucose-1-C^{14} is used (*136*). It should be pointed out that the equations devised by these authors are valid only if no drainage exists of intermediates of the pentose cycle for biosynthetic functions, a condition that cannot be fulfilled even in studies with resting cells. It is suspected that with organisms in which phosphogluconate cleavage amounts to a minor portion of glucose catabolized, this pathway may function mainly for the synthesis of pentoses, heptoses, and tetroses.

3. *The Radiorespirometric Method Based on Yields of* $C^{14}O_2$ *Observed in Time-Course Experiments*

The confusion that often arises from dealing with the uncontrollable dilution of isotopic substrates with unlabeled ones of endogenous origin prompted the development of method that is based on total yield of $C^{14}O_2$, without regard for specific activities (*137a*). In addition, the method developed by Wang and co-workers (*15*, *138*, *139*) permits a kinetic study of the oxidation sequences involved.

The radiorespirometric method for pathway studies calls for the collection as well as the determination of $C^{14}O_2$ produced at regular short intervals from a bank of concurrent experiments. [For convenience a new unit for measuring time is introduced here, called the relative time unit (RTU). This is defined as the time required to complete the initial catabolic reaction with respect to the administered substrate.* This unit is employed since rates of metabolism vary among organisms, and this device tends to relate the data from one organism to another more effectively.] Each represents the incubation of the test organism with a substrate labeled with C^{14} at a specific carbon atom. Currently, possible labelings are C-1 or C-2 of acetate; C-1, C-2, or C-3 of pyruvate; and C-1, C-2, C-3, C-3,4, C-6, or C-U of glucose or gluconate. These substrates represent all major carbon sources in the currently recognized catabolic pathways. Information collected in an experiment of this type is expressed as percentage radiochemical recovery of the labeled carbon atom in the respiratory CO_2 per unit time, and the kinetics of the process are automatically displayed when one plots the interval recoveries versus time. The cumulative $C^{14}O_2$ recovery, and the radioactivity in the medium of incubation or fermentation products from microorganisms as well as radioactivity in the organisms after incubation, also provide important information on the over-all assimilation of the administered substrate (*140*). Such patterns are also very useful in the elucidation of catabolic mechanisms.

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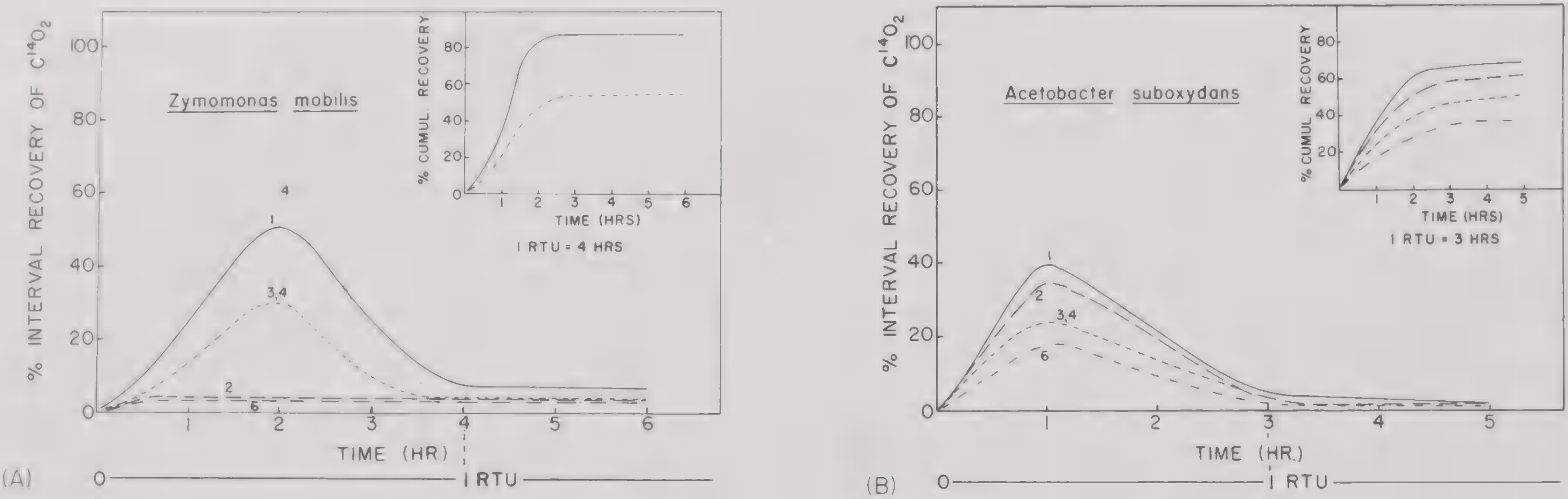
* For many organisms and substrates, this is equivalent to the time required for complete removal of the substrate from the medium. Sometimes this is difficult to diagnose, as in the conversion of gluconate to ketogluconate. Often the cessation of appearance of the initially oxidized carbon atom (e.g., C-1 or C-3,4) is used to denote the duration of 1 RTU. It should be stressed that the introduction of the RTU concept is made mainly to focus attention on the optimal duration of the experiment, when primary catabolic reactions tend to be completed. Variations in reaction rates among concurrent pathways are thus minimized.

It should be noted that the data based on interval $C^{14}O_2$ recoveries collected in the radiorespirometric experiment are related to the data on specific activity of $C^{14}O_2$ described under method no. 1 since CO_2 yields from organisms in concurrent experiments under the same conditions tend to be alike. The advantage in the use of the interval $C^{14}O_2$ yields lies in the fact that they are not affected by endogenous unlabeled material or other unlabeled carbon sources in the incubation medium, thus permitting one to trace the fate of a given carbon atom of a particular substrate in cellular respiratory functions. This is an important point if it is realized that the conventional respirometric method (e.g., with Warburg flasks) does not provide information on the respiratory activity of a given substrate, e.g., glucose, in the presence of other carbon sources such as yeast extract or peptone.

The kinetic feature of the radiorespirometric method permits one to examine the progress of an experiment if a rapid counting technique, such as liquid scintillation counting, is used for the assay of the radioactivity in the respiratory CO_2 (*141*). With proper experimental design, it is therefore possible to trace the complete biological fate of a given substrate, i.e., complete utilization of the administered substrate with respect to all catabolic pathways. This is important since the over-all catabolic picture so obtained will not suffer greatly from the variation in reaction rates when two or more concurrent catabolic sequences are under observation.

Illustrations of the data analysis used in the radiorespirometric method are given in Fig. 7, parts A, B, and C. Three patterns of glucose catabolism are represented by the three organisms chosen: *Zymomonas mobilis*, *Acetobacter suboxydans*, and *Bacillus subtilis*. Each represents a specific type, and the patterns of $C^{14}O_2$ evolution are characteristic of each type, as follows:

(*a*) The first of these characteristic responses is given by *Z. mobilis* (*142*). This organism, which reportedly possesses no Krebs cycle (*143*), shows a radiorespirometric pattern, given in Fig. 7A, in which C-1 and



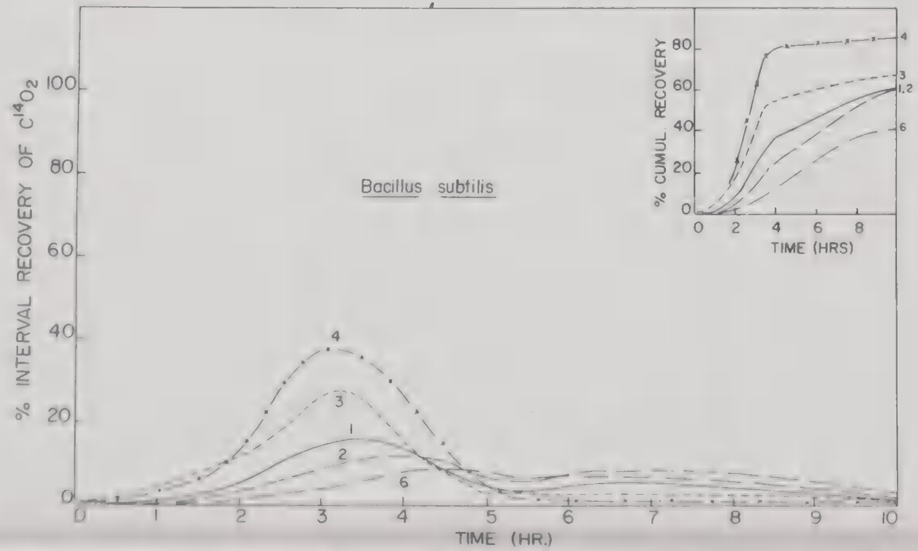


FIG. 7. Time course plots of radiochemical recoveries in CO_2 from metabolizing specifically labeled glucose. (A) *Zymomonas mobilis* (formerly called *Pseudomonas lindneri*). 1 RTU = 4 hours. (B) *Acetobacter suboxydans*. 1 RTU = 4 hours. (C) *Bacillus subtilis*. 1 RTU = 4.5 hours. In all systems, the following legends are employed: ———, glucose-1-C^{14}; — — —, glucose-2-C^{14}; - - - - -, glucose-3,4-C^{14}, •••••••, glucose-4-C^{14}, —•—•—•—•—, glucose-6-C^{14}. For the definition of the relative time unit (RTU) see the text. Insets: cumulative recoveries of $C^{14}O_2$.

The rate of utilization of glucose has been shown to be constant in microbial systems (*165*). Similar relations may not be realized in animal or plant tissue.

C-4 of glucose are converted to CO_2 extensively at the same rate. This is to be expected from operation of the Entner-Doudoroff pathway.

(*b*) *A. suboxydans* is one of the few microorganisms in which glucose appears to be catabolized exclusively via the pentose cycle. As with *Z. mobilis* above, the TCA cycle is reported not to be operative in this organism (*144*). As shown in Fig. 7B, one finds that the conversion of glucose carbon atoms to CO_2 follows the order of C-1 > C-2 > C-3,4 > C-6. It is noteworthy that although the over-all rates of $C^{14}O_2$ production from the labeled carbon atoms are essentially alike, the total recovery of each of the labeled carbon atoms in CO_2 is by no means the same. This indicates that, despite the fact that experiments were carried out under resting conditions, there was drainage of intermediates from the pentose cycle either for biosynthetic functions or as fermentation products. Extensive recycling of some of the glucose carbon atoms is therefore depressed.

(*c*) The radiorespirometric patterns for the utilization of glucose by *Bacillus subtilis* (*145*) is typical for organisms that seem to rely mainly on glycolysis (in conjunction with the Krebs cycle) and less significantly on phosphogluconate cleavage for carbohydrate catabolism (Fig. 7C). One can readily recognize two distinct phases displayed on the pattern, with the dividing line located approximately at 1 RTU (relative time unit). During the first phase, assimilation of substrate takes place predominantly. Glycolysis is clearly indicated by the high recovery of C-4 and C-3 of glucose in CO_2. Meanwhile, the occurrence of an alternate pathway is revealed by the observation that complete equivalence in metabolic behavior between C-3 and C-4 or C-1 and C-6 was not realized as called for by the exclusive operation of glycolysis (Fig. 8). The preferential conversion of C-1 over that of C-6 to CO_2 suggested that the alternate pathway involved is phosphogluconate decarboxylation. However, the pentose phosphate so formed evidently did not participate extensively in respiratory functions via the pentose phosphate cycle, otherwise $C^{14}O_2$ production from C-2 should approach or equal that of C-1. This is not surprising, inasmuch as cell proliferation occurred in this experiment, which would necessitate drainage of various intermediates for biosynthetic functions.

In the second phase of the radiorespirometric pattern [after exhaustion of administered glucose (or after 1 RTU)] one finds that the substrate carbon atoms, particularly C-6, C-2, and to some extent C-1, which were previously retained in the medium or incorporated into the cellular constituents, are converted to respiratory CO_2 at a significantly enhanced rate for a period of some hours. The order of $C^{14}O_2$ production during this phase, i.e., C-2 > C-6 > C-1 > C-3 or C-4, is in line with the contention that the depletable cellular constituents, perhaps amino acids, were oxidized mainly by glycolysis-Krebs cycle processes and their conversion to $C^{14}O_2$ reflects the principal respiratory mechanism, i.e., the TCA cycle.

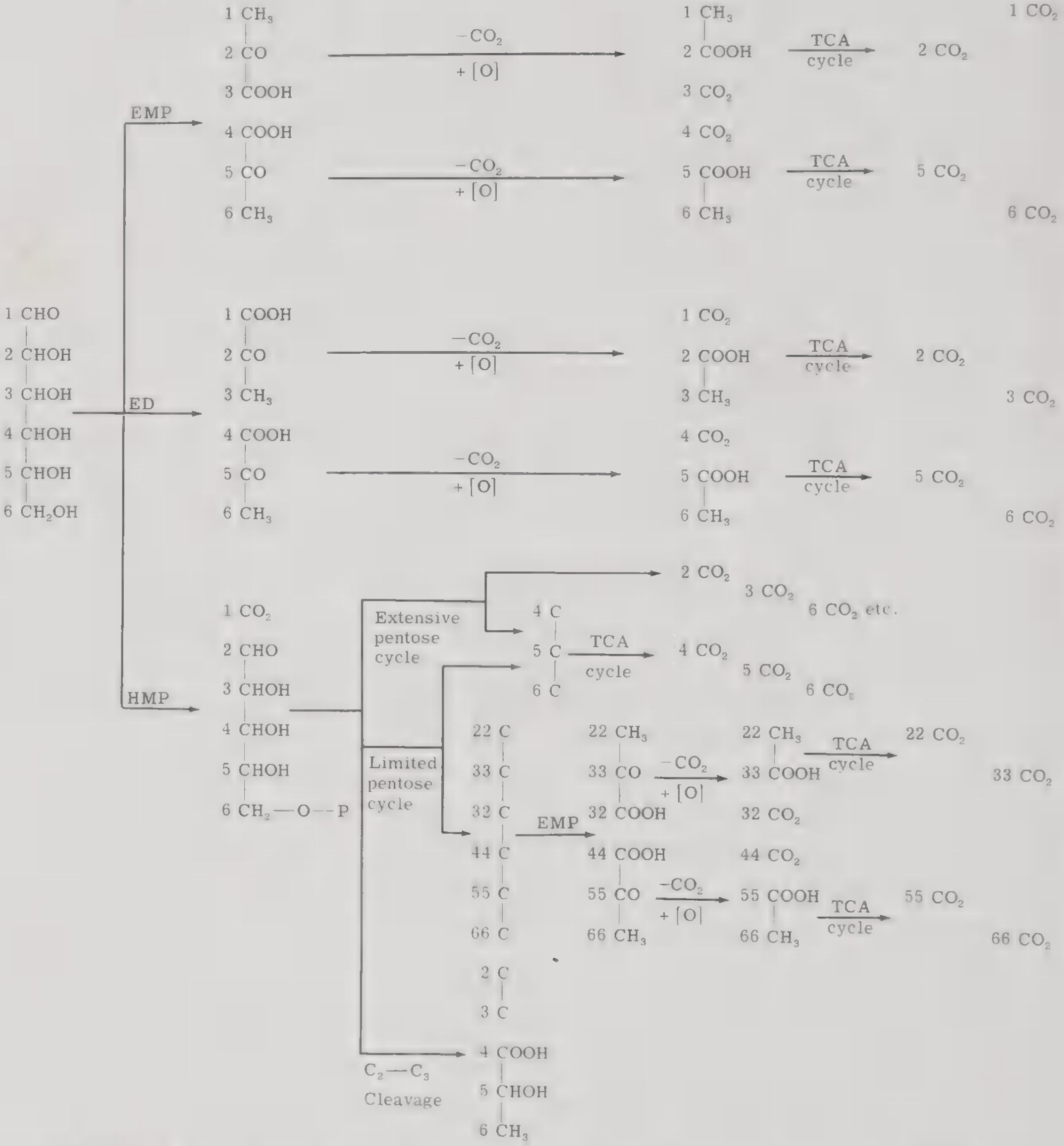


FIG. 8. Relative rates of CO_2 production from glucose via different pathways.

The usefulness of the radiorespirometric experiments is further illustrated by the data given in Fig. 9 on the utilization of gluconate by *B. subtilis* (*145*). The advantage of using C^{14} specifically labeled gluconate to trace the fate of pentose phosphate is obvious inasmuch as the direct utilization of gluconate via glycolysis (upon direct conversion of gluconate to glucose) seems impossible. This permits one to examine the utilization of gluconate by way of F-6-P upon the completion of one turn of the pentose cycle. It also permits the testing of one of the main implications of the paper by Katz and Wood, since these authors appear to imply that G-6-P traversing the pentose cycle can be further catabolized only via a repeated mechanism of the same nature, without side steps into other pathways via a common intermediate.

The radiorespirometric patterns given in Fig. 9 provide the following information: (*a*) gluconate is readily catabolized, presumably after initial phosphorylation; (*b*) the pathway responsible for the utilization of gluconate is neither the classic Entner-Doudoroff pathway nor a combination of the latter with the pentose cycle. In the Entner-Doudoroff pathway, one would expect the metabolic equivalence of C-1 with C-4, C-2 with C-5, and C-3 with C-6 (Fig. 8). In the present situation, the $C^{14}O_2$ recoveries from individual carbon atoms were such that there exist no similarites between the equivalent carbon atom within each of the pairs; (*c*) instead, gluconate appears to be catabolized via the following sequence:

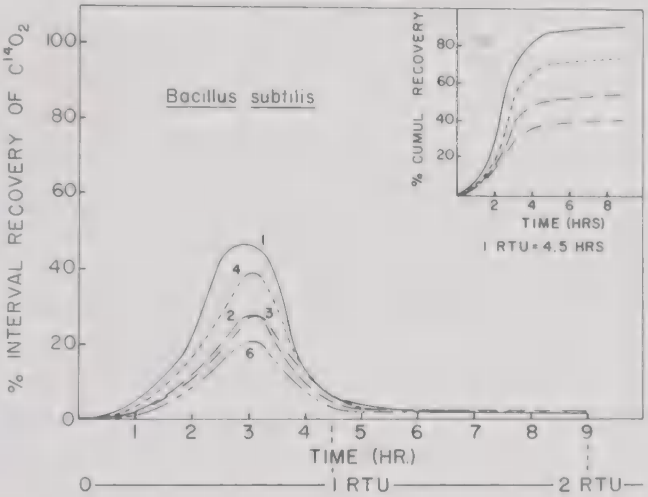
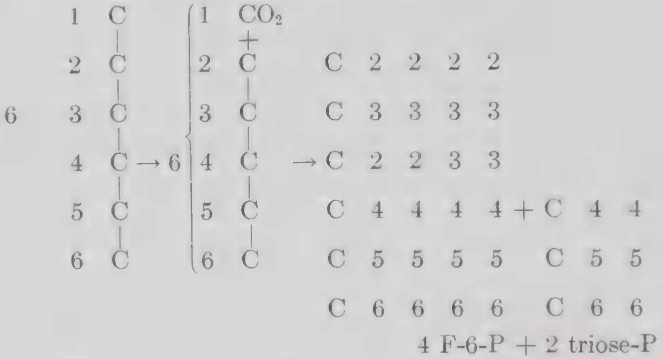


FIG. 9. Time-course recovery of $C^{14}O_2$ from specifically labeled gluconate in *Bacillus subtilis*. Inset: cumulative recoveries of $C^{14}O_2$.

$$\text{Gluconate-6-phosphate} \xrightarrow{\text{pentose cycle}} \text{Fructose-6-phosphate}$$

$$\text{Fructose-6-phosphate} — \begin{cases} \xrightarrow{\text{major}} \text{Fructose-1,6-diphosphate} \rightarrow \text{Glycolysis} + \text{TCA cycle} \\ \xrightarrow[\text{minor}]{} \text{Glucose-6-phosphate} \rightarrow \text{Pentose cycle} \end{cases}$$

The conclusion is drawn from the consideration that the fructose-6-phosphate derived from the labeled gluconate should bear the labeling patterns shown in Scheme 3 (*14, 146*) (see also Fig. 3):



(3)

Further catabolism of these fructose-6-P molecules may be predicted as follows:

Let us consider the fate of gluconate-C-2: In Table IV, data are given for the total yields of $C^{14}O_2$ from individual carbon atoms of glucose and gluconate. For gluconate-2-C^{14}, the fraction decarboxylated is 88%. *This represents the maximum amount of substrate gluconate that could have traversed the cycle* (and the probable actual amount that did so).

According to Beevers' scheme, the re-formed fructose-P (from gluconate) will have the original C-2 from gluconate in carbons A and C of the fructose; 67% of this will be in carbon A, 33% in carbon C.

If we now assume that the re-formed fructose-6-P behaves like ad-

TABLE IV

Percentage Recoveries of Individual Carbon Atoms in *Bacillus subtilis* Oxidations

Substrate	CO_2	Cells	Medium	Total
Glucose-1	60	22	13	95
Glucose-2	60	25	16	101
Glucose-3	67	19	16	102
Glucose-3,4	77	15	12	104
Glucose-4	87	11	8	106 (calc.)
Glucose-6	41	23	41	105
Gluconate-1	88	1	11	100
Gluconate-2	54	22	18	94
Gluconate-3	54	31	17	102
Gluconate-3,4	63	19	15	97
Gluconate-4	72	7	13	92 (calc.)
Gluconate-6	44	36	18	98

ministered *glucose*, the recovery of carbons A and C (from Table IV) will be 60 and 67%.

$$\begin{aligned} \text{Multiplying } 88\% \times 67\% \times 60\% &= 35\% \\ \text{and } 88\% \times 33\% \times 67\% &= 20\% \\ \textit{Total} &= 55\% \end{aligned}$$

calculated recovery of $C^{14}O_2$ from C-2 of gluconate.

This agrees well with the observed value of 54% from C-2 of gluconate (Table V). Formation of $C^{14}O_2$ from other positions in the gluconate molecule also agrees fairly well with predictions. A repeat experiment, not shown here, carried out 6 weeks later than the one listed in Table V, gave virtually identical results. There is strong indication that fructose-6-P exists in a general pool in this organism; this compound is attacked in the same manner whether it is re-formed from the pentose cycle or is administered as substrate glucose. The scheme of Katz and Wood, which would seem to imply that pentose cycle carbon is trapped and recycled until oxidized, would thus appear to require re-evaluation; the Embden-Meyerhof pathway, which in *B. subtilis* carries most of the "traffic," proceeds apace in the presence of phosphogluconate decarboxylation, and it is apparently unnecessary to make allowance for repeated recycling of pentose as these authors have done.

The foregoing experiment with *B. subtilis* provides experimental evidence for the assumption made by Dawes and Holms in connection with their study of pathway participation in *Sarcina lutea* (*136*). Although information of this type obtained in experiments with microorganisms cannot be applied directly to animal metabolism, it is reasonable to suggest that the fate of pentose phosphate, as derived from glucose via the pentose phosphate pathway, in animal metabolism should be carefully examined so that the importance of recycling of fructose-6-phosphate in the over-all glucose metabolism in animals can be better evaluated.

Quantitative Estimation of Pathways. Once the nature of the catabolic pathways operative in a biological system is recognized, the $C^{14}O_2$ yield data can be used to estimate the participation of individual pathways with respect to the over-all catabolism of glucose (*15*, *138*, *139*). The basic concepts underlying this method are derived mainly from the following considerations:

(*a*) The cumulative yields of respiratory $C^{14}O_2$ from the labeled carbon atoms of substrate observed at 1 RTU represent the over-all metabolic behavior of the entire catabolized substrate. This means that any difference in reaction rates inherent to individual catabolic pathways is minimized.

TABLE V

DATA ANALYSIS: *Bacillus subtilis* SYNTHETIC MEDIUM

Glucose skeleton	Gluconate carbon number	Fraction of gluconate decarbonated *A*	Fraction of relocated gluconate-C in glucose[c] *B*	Recovery of glucose-C in CO_2 at 10 hours *C*	Calculated $C^{14}O_2$ recovery (fraction) $A \times B \times C$	Calculated $C^{14}O_2$ recovery (total) (%)	Observed $C^{14}O_2$ recovery (%)
	Gluconate-2	0.88	0.67 in C-(A)	0.60	0.35	55	54
		0.88	0.33 in C-(C)	0.67	0.20		
(A) CHO							
	Gluconate-3	0.88	0.67 in C-(B)	0.60	0.35	55	54
(B) C		0.88	0.33 in C-(C)	0.67	0.20		
(C) C	Gluconate-4[a]	0.88	1 in C-(D)	0.87	0.77	77	72
(D) C	Gluconate-4[b]	0.88	0.83 in C-(D)	0.87	0.64	74	72
			0.17 in C-(C)	0.67	0.10		
(E) C							
	Gluconate-6[a]	0.88	1 in C-(F)	0.41	0.36	36	44
(F) CH_2OH	Gluconate-6[b]	0.88	0.83 in C-(F)	0.41	0.30	39	44
			0.17 in C-(A)	0.60	0.09		

[a] Assuming no triose recombination.

[b] Assuming triose recombination.

[c] According to scheme by Beevers (*14*).

(*b*) At 1 RTU, the $C^{14}O_2$ of the labeled substrate carbon atoms derived from a relatively direct pathway (such as the conversion of C-1 of glucose to CO_2 via phosphogluconate decarboxylation) represents the extent of "traffic" traversing this pathway. The radiochemical yields, unlike specific activities of $C^{14}O_2$, are not seriously affected by dilution of unlabeled substrate derived from regenerating mechanisms. To illustrate this, one can again use the conversion of C-1 of glucose via phosphogluconate decarboxylation as an example. The $C^{14}O_2$ yield from glucose-1-C^{14} via phosphogluconate cleavage should remain the same, despite the fact that the substrate glucose may have been diluted at the glucose-6-phosphate stage by fructose-6-phosphate produced from substrate glucose via the pentose cycle reactions, or by the fructose-1,6-diphosphate produced via the recombination of trioses via the reversed aldolase reaction.

(*c*) The metabolic nonequivalence observed at 1 RTU with a given set of labeled carbon atoms of the substrate, i.e., C-1 and C-6 of glucose in the concurrent operation of the glycolytic and the HMP pathway (Fig. 7C, for example) reflects quantitatively the participation of pathways.

With these basic considerations in mind, one can then derive equations for the estimation of pathway on the basis of the following assumptions:

(1) Consider that glucose may be catabolized in a biological system via two pathways: one yields two C_3 units and the other yields CO_2 and a C_5 unit. The former applies either to glycolysis or the Entner-Doudoroff pathway. (There exists no evidence at the present time that these two pathways are operative concurrently in a given biological system.) The latter type refers to phosphogluconate cleavage.

(2) The preferential conversion of C-1 of glucose to CO_2 via phosphogluconate decarboxylation is a rapid and virtually irreversible process.

(3) The C_3 units formed in either glycolysis or the Entner-Doudoroff pathway, best represented as pyruvate (Fig. 8), are decarboxylated promptly. It is understood that a small fraction of pyruvate may participate in CO_2 fixation processes, however, the magnitude of such processes is likely to be insignificant.

(4) The C_3 units (i.e., trioses and pyruvate) formed in glycolysis or the Entner-Doudoroff pathway are virtually equivalent to each other at the pyruvate stage with respect to further metabolic reactions.

(5) The formation of hexose by way of a recombination of trioses is insignificant in magnitude.

(6) The randomization of the hexose skeleton via transketolase ex-

change reaction or other similar processes (*130*) does not occur to any significant extent.

Most of the foregoing assumptions can be readily verified; this has been done, in at least one biological system (*139*) by separate experiments. It should be stressed that in studying the catabolism of a biological system under normal physiological conditions, such as intent plants, intact animals, or proliferating microorganisms, it is justifiable to believe that the catabolic "traffic" associated with a normal substrate is largely unidirectional toward sequential breakdown leading to the eventual formation of CO_2. Consequently, the randomization of labeling pattern in the substrate through the operation of reversed catabolic reactions is greatly minimized.

(7) The catabolic behavior of substrate gluconate is the same as that of gluconate derived from glucose via the HMP pathway.

On the basis of the foregoing assumptions, use can be made of the yields of $C^{14}O_2$ at 1 RTU from C^{14} specifically labeled glucose to estimate the pathway participations in the over-all catabolism.

Let G_1, G_2, G_3, G_4, $G_{3,4}$ and G_6 = percent radiochemical yield of substrate activity in the respiratory CO_2, from systems metabolizing equal amounts of glucose labeled with C^{14} at C-1, C-2, C-3, C-4, C-3 (or 4), or C-6, respectively, taken at 1 RTU, at which time the initial breakdown of administered substrate has been completed. These are expressed as fractions of unity.

A_1, A_2, A_3, A_4, $A_{3,4}$, A_6 = percent $C^{14}O_2$ yields from systems metabolizing equal amounts of gluconate labeled with C^{14} at C-1, C-2, C-3, C-4, C-3,4, or C-6 respectively, taken at 1 RTU, expressed as fractions of unity.

G_T = total radioactivity of each labeled substrate administered (always taken as 100%, or unity).

G'_T = fraction of the labeled substrate administered engaged in anabolic processes.

G_p = fraction of the administered glucose engaged in catabolic functions routed via phosphogluconate cleavage.

G_e (Embden-Meyerhof) = fraction of the administered glucose engaged in catabolic functions routed via glycolysis.

G_{ed} = fraction of the administered glucose engaged in catabolic functions routed via the Entner-Doudoroff pathway.

The equations for pathway estimations are given as follows:

(1) With biological systems that catabolize substrate glucose extensively, such as microorganisms, and under the additional assumption that the pentose phosphate formed in the HMP pathway does not participate in CO_2 production.

(*a*) With the concurrent operation of glycolysis and phosphogluconate decarboxylation;

$$G_p = \frac{G_1 - G_6}{G_T - G'_T} \tag{1}$$

$$G_e = 1 - G_p \tag{2}$$

When the magnitude of G'_T is small, Eq. 1 becomes

$$G_p = G_1 - G_6 \tag{3}$$

(*b*) With the concurrent operation of the Entner-Doudoroff pathway and phosphogluconate cleavage,

$$G_p = \frac{G_1 - G_4}{G_T - G'_T} \tag{4}$$

$$G_{ed} = 1 - G_p \tag{5}$$

When the magnitude of G'_T is small, Eq. 5 becomes

$$G_p = G_1 - G_4 \tag{6}$$

Inasmuch as there exists definite evidence that, at least in part, the pentose phosphate formed in the pentose pathway can be converted to CO_2, the foregoing equations, therefore, represent minimal limiting cases. $C^{14}O_2$ also originating from C-6 of glucose via the pentose pathway should not be included in the G_6 term given in Eqs. 1 and 3. With this consideration in mind, a correction factor for the G_6 term can be readily applied if use is made of $C^{14}O_2$ yield data in a gluconate-6-C^{14} experiment. This is possible since it is known that gluconate is not likely to be converted to glucose to any appreciable extent, a fact making it possible to trace the fate of C-6 glucose in the HMP pathway by labeling the corresponding carbon atoms in gluconate. Upon applying such a correction, one finds that:

(*c*) with the concurrent operation of glycolysis and phosphogluconate cleavage

$$G_p = \frac{G_1 - [G_6 - A_6G_p]}{G_T - G'_T} \tag{7}$$

And, when the magnitude of G'_T is small, Eq. 7 becomes

$$G_p = G_1 - [G_6 - A_6G_p] = \frac{G_1 - G_6}{1 - A_6} \tag{8}$$

Here again the participation of the glycolytic pathway can be calculated by subtracting the value of G_p from unity. The term G'_T can be ignored if there is reason to believe that the anabolic utilization of substrate glucose is not playing a significant role. If necessary, the magnitude of the term G'_T can be evaluated by an examination of the amount of radioactivity incorporated into cellular constitutents in experiments using glucose-3,4-C^{14}, glucose-3-C^{14}, or glucose-4-C^{14} (data on C-4 of glucose can be obtained indirectly from those in the glucose-3-C^{14} and glucose-3,4-C^{14} experiments) as test substrates.

Unfortunately, similar corrections for the G_4 term cannot be readily applied since a common intermediate, phosphogluconate, is shared by both the pentose phosphate pathway and the Entner-Doudoroff pathway.

(2) With biological systems that catabolize substrate glucose only to a limited extent, owing either to an extensive dilution of the labeled substrate by endogeneous materials of a similar nature or the diffusion of the labeled substrate into physiological compartments having relatively low metabolic activity, the exact amount of substrate glucose actually engaged in catabolism would be rather difficult to determine directly. This is indeed one of the major problems encountered when one wishes to study pathway participation in such biological systems as intact animals and intact plants.

Despite the complexity of a problem of this nature, a close approximation of the value $(G_T - G'_T)$ can be made by the use of Eq. 9 or Eq. 10, provided there is not significant accumulation of trioses or pyruvate or similar compounds from the administered glucose:

(*a*) with concurrent operation of glycolysis and the HMP pathway,

$$G_T - G'_T = G_1 - [G_6 - A_6 G_p] + [G_3 - A_3 G_p] \tag{9}$$

(*b*) with the concurrent operation of the Entner-Doudoroff pathway and phosphogluconate decarboxylation,

$$G_T - G'_T = G_1 \tag{10}$$

It should be stressed that Eqs. 1–10 are derived from current understandings of glucose catabolism and are hence subject to the validity of the assumptions set forth for their derivation. The picture of pathway participation in a given system observed with the radiorespirometric method is a close approximation of the catabolic organization of the system under the defined experimental conditions. Understanding of this nature is important in probing the function of an individual pathway in the over-all cell catabolism. In fact, regardless of the equations that are used to examine the participation of pathways in cellular catabolism, numerical values of pathway estimation are generally presented in a semiquantitative sense. Attachment of too great quantitative significance to these values is probably unsafe, especially in view of the limited information presently available on over-all catabolism and the

TABLE VI

ESTIMATION OF CATABOLIC PATHWAYS OF GLUCOSE

	Pathway estimation[a]				
Microorganism	EMP (%)	HMP (%)	ED (%)	Method	Reference
Acetobacter suboxydans	—	100	—	Radiorespirometry	*144*
Zymomonas mobilis	—	—	100	Radiorespirometry, Eq. 6	*142*
Pseudomonas saccharophila	—	—	100	Radiorespirometry, Eq. 6	*142*
Pseudomonas reptilivora	—	28	72	Radiorespirometry, Eq. 6	*142*
Pseudomonas aeruginosa	—	29	71	Radiorespirometry, Eq. 6	*142*
Sarcina lutea	70	30	—	Radiochemical yield of intermediate	*135*
Streptomyces griseus	97	3	—	Radiorespirometry, Eq. 3	*15*
Penicillium chrysogenum	77	23	—	Radiorespirometry, Eq. 3	*15*
Penicillium digitatum				Radiorespirometry:	
	83	17	—	Eq. 3	*15*
	77	23	—	Eq. 2	*146*
Fusarium lini	83	17	—	Sp. Act. of $C^{14}O_2$	*147*
Escherichia coli	72	28	—	Radiorespirometry, Eq. 3	*15*
Saccharomyces cerevisiae	88	12	—	Radiorespirometry, Eq. 3	*15*
	83–100[b]	0–27[b]	—	Sp. Act. of intermediates	*132*
Candida utilis	66– 81	19–34	—	Sp. Act. of intermediates	*132*
Bacillus subtilis				Radiorespirometry:	
	74	26	—	Eq. 3	*145*
	59	41	—	Eq. 7[c]	

[a] EMP = Embden-Meyerhof-Parnas pathway (glycolysis); HMP = hexose monophosphate pathway (phosphogluconate cleavage); ED = Entner-Doudoroff pathway.

[b] Experimental conditions varied from depleted resting cells to growing cells.

[c] Equation 7 is used under the assumption that $G'_T = 0$.

possible variation of pathway participation in a given system under different physiological environments. A measure of the *approximate* contributions of various pathways to total carbohydrate breakdown has been made in Table VI, using some of the refinements outlined in the foregoing pages; as a single example, the estimated participation of phosphogluconate rises from 26 to 41% when the refinements contained

TABLE VII

PATHWAY PARTICIPATIONS IN MICROBIAL CATABOLISM OF GLUCOSE

Microorganism	Pathway participation[a]		Reference
	Major	Minor	
Zymomonas mobilis	ED	—	*142, 148, 149*
Pseudomonas fluorescens	ED	HMP	*133, 142, 150, 151*
Pseudomonas saccharophila	ED	—	*114, 142*
Pseudomonas aeruginosa	ED	HMP	*142*
Pseudomonas viridilivida	ED	HMP	*152*
Acetobacter aceti	HMP	—	*153*
Acetobacter suboxydans	HMP-PC	—	*144*
Acetobacter industrius	HMP-PC	—	*154, 155, 156*
Acetobacter melanogenus	HMP-PC	—	*154, 155, 156*
Acetobacter xylinum	HMP	—	*154*
Azotobacter vinelandii	HMP-PC	—	*53, 157, 158*
Azotobacter chroococcum	HMP-PC	—	*158*
Zygorrhynchus moelleri	EMP	HMP	*159*
Streptomyces griseus	EMP	HMP	*160*
Penicillium chrysogenum	EMP	HMP	*15, 161, 162*
Penicillium digitatum	EMP	HMP	*15, 146*
Fusarium lini	EMP	HMP	*147*
Aspergillus niger	EMP	HMP	*15, 74, 163*
Verticillium albo-atrum	EMP	HMP	*164*
Sarcina lutea	EMP	HMP	*135, 136, 137*
Saccharomyces cerevisiae	EMP	HMP	*15, 132, 165, 166, 167*
Candida utilis	EMP	HMP	*132*
Escherichia coli	EMP	HMP	*15, 168*
Bacillus subtilis	EMP	HMP	*15, 169*
Aerobacter aerogenes	EMP	HMP	*145, 170, 171*
Micrococcus radiodurans	EMP	HMP	*171, 172, 173*
Asterococcus mycoides	EMP	—	*174, 175*
Pasteurella pestis	EMP	HMP	*176, 177*
Propionibacterium arabinosum	EMP	—	*178*
Propionibacterium shermanii	EMP	—	*178*
Clostridium perfringens	EMP	—	*179*
Clostridium thermoaceticum	EMP	—	*180*
Butyribacterium rettgeri	EMP	—	*181*
Leuconostoc mesenteroides	HMP	—	*171, 182*
Leuconostoc dextranicum	HMP	—	*171*
Lactobacillus pentoaceticus	HMP	—	*171*
Lactobacillus fermenti	HMP	—	*171*
Lactobacillus pentosus	EMP	—	*171*
Streptococcus faecalis	EMP	—	*171*
Lactobacillus casei	EMP	—	*183*

[a] EMP = Embden-Meyerhof-Parnas pathway (glycolysis); ED = Entner-Doudoroff pathway; HMP = hexose monophosphate pathway (phosphogluconate cleavage); HMP-PC = hexose monophosphate-pentose cycle pathway.

in Eq. 7 are applied. The data in Table VII are less certain quantitatively, hence the terms "major" or "minor" are applied as appropriate.

With regard to the catabolism of glucose and allied carbohydrates in animals, it appears that glucose is catabolized mainly by way of glycolysis, whereas phosphogluconate cleavage may participate to a minor, variable extent (*184*). Examples may be cited of the qualitative experiments carried out by Bloom (*129*) with a great variety of rabbit tissues including brain, kidney, heart, spleen, liver, bone marrow, and testes; the estimation of glucose pathways in rat tissue (*126*, *127*, *185*), intact rats (*186*), mammary gland of rats (*125*), blood cells (*3*), insects (*187*), cow's udder (*188*), retina (*189*), carp (*190*), etc. Interesting side reactions have been revealed; the occurrence of the pentose cycle as well as the extent of recycling of hexose phosphate in cows were examined by Wood (*191*). By studying the isotopic distribution patterns of amino acids, glycerol (in the fat portion), and glucose isolated from milk derived from cows fed with specifically labeled substrate, it was possible to detect the transaldolase exchange reaction and enable the author to examine the possible effect of side reactions upon the estimation of pathways of glucose catabolism in biological systems.

As discussed in Section V,B on the role of the pentose cycle, the close relationship between the photosynthetic cycle and the pentose cycle in plants leads one to suspect that the latter pathway should play a role in the over-all hexose catabolism of plants. The operation of the pentose pathway has been demonstrated in corn coleoptiles, pea seedlings, and other tissues (*14*, *51*, *128a*, *192*, *193*, *194*) using C^{14} specifically labeled hexoses. The radiorespirometric method has been used to estimate the participation of the pentose phosphate pathway in tomato and pepper fruits (see references *138*, *139*, and *195*); the findings indicated that glycolysis prevails in these fruits, with the pentose phosphate pathway operating to an extent of only 20–30%. In germinating pea seeds (*196*, *197*) radiorespirometric examination has revealed that glucose is catabolized almost exclusively by glycolysis, with phosphogluconate cleavage estimated to be not more than 5% of the total metabolized glucose. The participation of various glucose breakdown routes in leaf tissues has not yet been thoroughly studied. From current information, it appears that the catabolism of glucose in the plant relies heavily on the operation of the EMP pathway. Here again fructose-6-phosphate can be derived from glucose by way of the pentose cycle reactions; however, judging from the findings with tomato fruit utilizing C^{14} specifically labeled gluconate (*139*), the regenerated fructose-6-phosphate may have participated in further catabolism in a manner similar to that of fruit glucose, i.e., mainly by way of glycolysis (after initial formation of hexose phosphate), instead of engaging extensively in recycling. It is of interest to note that the operation of the Entner-Doudoroff pathway has not yet been demonstrated to be operative in plant tissues.

In microorganisms, including bacteria and fungi, catabolism of glucose and its degradation products has been studied much more extensively than in either plants or animals. In fact, many of the metabolic pathways were first recognized from findings using microorganisms as test systems. This is perhaps not surprising in view of the fact that microorganisms show characteristically vigorous metabolic rates. A notable example was the work on the metabolism of glucose in the pseudomonads which led to the discovery of the Entner-Doudoroff pathway for glucose degradation (*114*).

Examination of the existing literature prompts the suggestion that several microorganisms may be classified (more so than previously has been done) on the basis of their respective metabolic behaviors. Thus, studies with pseudomonads, using the radiorespirometric technique, have revealed that various species in this category are equipped to catabolize glucose by way of the Entner-Doudoroff pathway either exclusively or combined with the pentose cycle (*142*). Similarly one finds that in the related *Acetobacter* species examined (*144, 154*) the pentose cycle plays a predominant or exclusive role in hexose metabolism. Even more interesting is the fact that such organisms as bakers' yeast, *Escherichia coli,* and *B. subtilis* show strong resemblance to each other in the manner in which they attack carbohydrates. In each organism, one can recognize easily the assimilation and depletion phases: during assimilation while primary breakdown of glucose takes place, glycolysis plays a preponderant role, with phosphogluconate cleavage occurring to a limited extent. The predominance of the TCA cycle is revealed in the depletion phase, $C^{14}O_2$ being produced in the order C-2 > C-6 > C-1 > C-3,4 from glucose.

Work done on the biosynthetic mechanisms for amino acids in microorganisms (*198–200*) has indicated the importance of the TCA cycle in the production of carbon skeletons of numerous varieties. To maintain the continuous operation of the TCA cycle, however, it is mandatory that one or more of the key members of the cycle be furnished from an independent mechanism. The elucidation of such mechanisms has attracted considerable interest from a number of research workers. Previously, the fixation of CO_2 in a $C_3 + C_1$ or a $C_1 + C_2 + C_1$ condensation (both may take place via a number of possible mechanisms) have been recognized as playing an important role (*201–207*). More recently the elucidation of the exact nature of the glyoxalate cycle (*208*), which consists of the combined operation of the isocitratase and malate synthetase reactions (*209, 210*), led workers to believe that this could be important in the production of C_4 acids which are in turn fed into the TCA cycle for biosynthetic functions. In any event, it is not surprising to find that with such organisms as *Zymomonas mobilis,* which rely on external supplies of amino acids for growth, glucose catabolism involves only those pathways that will convert glucose to C_3 or C_2 compounds such as lactic acid and ethanol.

B. POSSIBLE ROLE OF THE PENTOSE CYCLE IN METABOLISM

The exact role of the pentose cycle is not yet clear. The ready reversibility of the enzymes of the cycle, particularly in the nonoxidative portion, raises the possibility that various intermediates may be synthesized from glucose by the pentose cycle, and further that these syntheses may be accomplished by *reversal* of the scheme, i.e., through the use of transketolase and transaldolase. Aside from the obvious need for ribose and deoxyribose in synthesis (*211, 212*) as well as for shikimic acid (*213*), one such example involves the transport of carbon in photosynthesis, where a close relative of the cycle, ribulose 1,5-diphosphate, serves as a primary acceptor of CO_2 (*214, 215*). The reactions that follow the incorporation of CO_2 involve both pentose cycle and glycolysis intermediates, en route to stored carbohydrate in the plant. Scheme 4 is a summary of the reactions that have been suggested (*216*).

$$\begin{aligned}
3\text{ Pentose-P} + 3\text{ATP} &\rightarrow 3\text{Ru-di-P} + 3\text{ADP}\\
3\text{Ru-di-P} + 3CO_2 + 3H_2O &\rightarrow 6\text{ P-glycerate}\\
6\text{ P-glycerate} + 6\text{ATP} &\rightarrow 6\text{di-P glycerate} + 6\text{ADP}\\
6\text{di-P glycerate} + 6\text{TPNH} + 6H^+ &\rightarrow 6\text{GLA-P} + 6\text{TPN}^+ + 6PO_4^{3-}\\
2\text{GLA-P} &\rightarrow 2\text{DHA-P}\\
2\text{GLA-P} + 2\text{DHA-P} &\rightarrow 2\text{F-1,6-di P}\\
2\text{F-1,6-di P} + 2H_2O &\rightarrow 2\text{F-6-P} + 2PO_4^{3-}\\
\text{F-6-P} + \text{GLA-P} &\rightarrow \text{Xu-5-P} + \text{E-4-P}\\
\text{F-6-P} + \text{E-4-P} &\rightarrow \text{SH-7-P} + \text{GLA-P}\\
\text{SH-7-P} + \text{GLA-P} &\rightarrow 2\text{ Pentose-P}
\end{aligned}$$

$$\textit{Sum:}\ 3CO_2 + 9\text{ATP} + 5H_2O + 6\text{TPNH} + 6H^+ \rightarrow \text{GLA-P} + 9\text{ADP} + 6\text{TPN}^+ + 8PO_4^{3-} \quad (4)$$

Although Scheme 4 is speculative, and some steps may not be strictly correct (*217*), the over-all import of the suggested scheme is one of reversal of pentose cycle reactions during photosynthesis.

A second example dealing with reversal of the cycle involves the synthesis of ribose in nucleic acids in the chick. Bernstein (*218*) fed chicks labeled acetate, glycine, or formate, then analyzed for C^{14} in tissue ribose (in RNA). He concluded that the *oxidative* pentose cycle could not account for the labeling observed. However, Horecker and Mehler (*219*) recalculated the results on the assumption that the ribose had arisen via *reversal* of the nonoxidative portion of the pentose cycle and found excellent agreement with the expected values.

A third example, pointing to the importance of the cycle in a synthetic role, comprises the finding (*32*, *220*) that the pentose cycle enzymes are more active and/or abundant during mammalian fetal development, and that in the final one-fourth to one-third of the gestation period, the fetus develops "adult" characteristics with respect to carbohydrate breakdown, i.e., the glycolysis-Krebs cycle route is greatly preferred in the later stages as well as in postpartum life. These examples support the idea that the pentose cycle may serve in many organisms mainly as a pathway for synthesis, even though *A. suboxydans* (*144*) and a few related microorganisms may under appropriate conditions use this pathway oxidatively, for carbohydrate breakdown. The extent to which oxidative metabolism of carbohydrates may be routed via the pentose cycle will depend upon the simultaneous utilizability of other pathways.

Finally, as suggested in the review by Horecker and Hiatt (*2c*), a fourth possible role of the pentose cycle (here operating clockwise, or in the forward direction) may be to supply TPNH to the system. In many species, the pentose cycle is TPN-specific; glycolysis and Krebs cycle oxidations are, on the other hand, largely DPN-requiring (a possible exception in some systems is isocitric dehydrogenase) so that processes such as fatty acid synthesis may derive significant amounts of needed TPNH from phosphogluconate cleavage.

It can, therefore, be stated that so far as the total metabolism of hexoses is concerned, i.e., both respiration and synthesis, glycolysis, particularly in conjunction with a complementary route such as the TCA cycle, appears still to be quantitatively a most important avenue in biological systems.

VI. Direct Oxidations of Glucose and Polyols

A. DIRECT OXIDATION OF GLUCOSE

The direct oxidation of glucose yields gluconic acid. The latter diverts to a number of pathways, as shown in Fig. 10. Some of these have been discussed in previous sections. Here emphasis will be made only on these nonphosphorylative reactions.

The first step in the oxidation of glucose to gluconic acid is through gluconolactone. Two different classes of enzymes have been found: one is linked with flavin and the other with pyridine nucleotide. The former is usually called glucose oxidase and the latter glucose dehydrogenase.

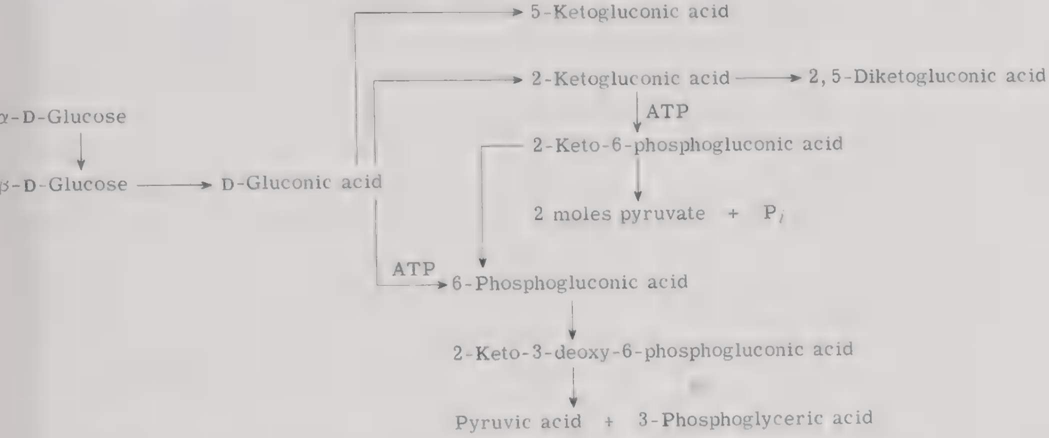


FIG. 10. Direct oxidation of glucose and multiple pathways of D-gluconic acid oxidation.

The lactone formed from either enzymatic reaction may be hydrolyzed enzymatically or nonenzymatically. The reaction sequence (*i–iii*) may be represented as follows:

(*i*) $$\begin{array}{l}\beta\text{-D-Glucose} \\ \delta\text{-D-Gluconolactone}\end{array} \circlearrowleft\circlearrowright \begin{array}{l}\text{DPNH (TPNH)} + H^+ \\ \text{DPN}^+ \text{ (or TPN}^+\text{)}\end{array}$$

(*ii*) $$\begin{array}{l}\beta\text{-D-Glucose} \\ \delta\text{-D-Gluconolactone}\end{array} \circlearrowleft\circlearrowright \begin{array}{l}\text{FADH}_2\text{-enzyme} \\ \text{FAD-enzyme}\end{array} \circlearrowleft\circlearrowright \begin{array}{l}H_2O_2 \\ O_2\end{array}$$

(*iii*) $$\delta\text{-D-Gluconolactone} + H_2O \rightarrow \text{Gluconate}^- + H^+$$

In both sequences the lactone formed from either the pyridine nucleotide or the flavin-linked enzyme is most probably in the δ-configuration. Thus in this aspect the enzymatic oxidation follows the same pattern as the chemical oxidation.

Thermodynamic considerations (the standard free energy change is about 2 kcal.) would suggest that Reaction (*i*) is reversible. Indeed it has been found (*221, 222*) that the equilibrium constant, $K =$ (gluconolactone) (DPNH)/(glucose) (DPN$^+$) is 15 at pH 6.7 (the temperature was not given but was presumably about 30°). Actually, most of the energy released in the over-all reaction comes from the neutralization of gluconic acid (*221*). It is doubtful that the reaction *per se* can be considered as a functional energy-yielding process, but the reduced coenzyme formed may possibly go through the respiratory chain.

The flavin-linked dehydrogenase or oxidase undergoes a different category of reaction as far as release of energy is concerned. Since molecular oxygen is involved, the standard free energy change for Reaction *ii* must be about —30 kcal.; for the over-all oxidation from glucose to gluconic acid and hydrogen peroxide it would be —33.6 kcal. It is truly an exergonic reaction. However, it is not certain whether the reaction even *in vivo* can be coupled with other reactions so that the energy given can be functionally conserved into ATP.

In general both the oxidase and the dehydrogenase catalyze the oxidation of nonphosphorylated sugars. Although they occur widely in microbial phyla, they do not appear to play as important a role in metabolism as enzymes in other well-known cycles. It would be fair to say, however, that the apparent lack of importance may be due to the deficiency in understanding these systems.

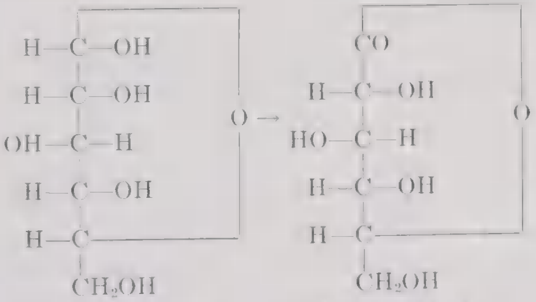
Müller (*223–225*) as early as 1928 described a glucose oxidase in *Aspergillus niger* and *Penicillium glaucum*. Later an apparently similar enzyme was found in other species of *Aspergillus* (*226*) and in *Fusarium lini* (*227*). However, Müller (*228*) as well as Ogura (*229*) has claimed that two different enzymes exist in mold. One reacts with oxygen and the other does not. Müller's preparations catalyze the oxidation of glucose by molecular oxygen, and are cyanide insensitive. Their specific activities are actually very low. Franke and co-workers (*230, 231*) have been able to purify the enzyme further. They have found hydrogen peroxide as a product and a flavin as the coenzyme. Using the preparation with Q_{O_2} value of 8000 at 30°, Franke (*232*) has challenged the claim made by Müller and by Ogura as far as *A. oryzae*, *A. niger*, and *P. glaucum* are concerned.

In the early 1940's an English group energetically worked on an antibiotic, notatin (*226, 233*). They prepared samples of about 90% purity as measured by electrophoresis and ultracentrifugation. Using this sample Keilin and Hartree (*234*) have conclusively resolved the dispute between Müller and Franke. Samples with Q_{O_2} values as high as 30,000 react also effectively with 2,6-dichlorophenol indophenol. At the same time the identity of the glucose oxidase of Müller and Franke, notatin of Raistrick *et al.*, and penicillin B of van Bruggen *et al.* (*235*) has been definitely proved.

The absorption maxima of notatin, according to Keilin and Hartree, are at 377 and 455 mμ whereas those of penicillin B are at 375 and 452 mμ. The difference is evidently due to the difference in operation. From the flavin content the molecular weight of the enzyme is calculated at 83,000 (*234*), but that derived from physical determinations is 152,000 (*236*). It is suggested that each enzyme contains two prosthetic groups. The flavin can replace the coenzyme of D-amino acid oxidase, and thus it is flavin adenine dinucleotide. It is interesting to note that the enzyme does not show fluorescence within the pH range 2–8. The activity of the enzyme is parallel to its bactericidal action, and both are inactive beyond this pH range. In fact it is now known that the bactericidal action is from an enzymatic product, hydrogen peroxide (*234*).

A long list of specially purified sugars and sugar derivatives has been tested for specificity of the enzyme. Only 6-methyl glucose and 4,6-dimethyl glucose show about 2% of the glucose activity; mannose, xylose, trehalose, maltose, galactose, melibiose, and cellobiose possess less than 1% of the activity toward glucose, whereas all others are inactive (*234*, *237*). In fact, this enzyme has been used as a specific agent in the microdetermination of glucose (*238*). Recently, however, it has been found that 2-deoxy-D-glucose reacts actively (*239*) to give 2-deoxy-D-gluconic acid (*240*).

Glucose oxidase catalyzes the oxidation of the β sugar much faster than it does the α variety (*237*, *241*). Here again it resembles the chemical oxidation; β-glucose is oxidized by bromine about 35 times more rapidly than the α isomer (*242*, *243*). It has been postulated (*244*, *245*) that the ring form of glucose, but not the free carbonyl group, is directly oxidized as shown (I, II).



Glucopyranose (I) Glucono-δ-lactone (II)

The oxidative product from both chemical and enzymatic reactions is the δ-lactone. This fact may serve as indirect evidence to show that the sugar is mainly in the pyranoid form. However, the ratio of the enzymatic activities varies with different preparations. According to Keilin and Hartree (*237*) the mutarotation of glucose is catalyzed by a nondialyzable, thermolabile, highly active substance. The name mutarotase is given for this catalyst. Different preparations of notatin are contaminated with varied amounts of mutarotase. Other behaviors also support the suggestion of two entities rather than one enzyme species. For example, treatment of enzyme preparations at pH 9.1 for 20 minutes destroys nearly 90% of the oxidase activity but hardly affects the mutarotase. In fact, the two enzymes can be physically separated by ammonium sulfate fractionation and differential adsorption on calcium phosphate gel. The activity of mutarotase is unusually high. Its catalytic coefficient, 2×10^5 min.$^{-1}$, is higher than the coefficient of hydroxyl ion, 8.8×10^3. The latter is one of the most active nonenzymatic catalysts known for mutarotation (*246*).

Glucose oxidase has been also found in the red alga *Irridophycus* (*247*), in *Pseudomonas fluorescens* (*55*), and in *Azotobacter vinelandii* (*248*). The latter is especially interesting. The oxidation of glucose catalyzed by a partially purified particulate fraction from *A. vinelandii* accumulates D-glucono-δ-lactone almost quantitatively even in the absence of a lactone trapping agent. However, the nature of the prosthetic group of the enzymes from the alga and the pseudomonad has still not been clearly shown.

Glucose dehydrogenase was first found in the acetone powder of mammalian liver (*249, 250*). Even the most potent sample of the original preparations (*251*) is far from pure, since it slowly reacts with methylene blue. An earlier claim that DPN is the coenzyme (*252*) has been disputed by Das, who has found both DPN and TPN effective (*253*). Work on purification has also been done by Wainio and coworkers (*254, 255*). However, Brink (*222*) and Strecker and Korkes (*221*) have done the most extensive characterization. More recently it has been found that the purified preparation can use either pyridine nucleotide as coenzyme. The Michaelis constant of the glucose dehydrogenase from liver for TPN is smaller than that for DPN.

The H from C-1 of glucose is directly transferred to the 4 position of the nicotinamide of DPN. Like other dehydrogenases the enzyme also exhibits stereospecificity of the β-type; it involves the hydrogen transfer to the opposite side of the ring from that utilized in the reactions by yeast alcohol dehydrogenase and muscle lactic dehydrogenase or wheat germ malic dehydrogenase (*256, 257, 258*). In contrast to liver alcohol dehydrogenases, the absorption maximum of DPNH at 340 mμ does not shift to 325 mμ when it is linked with apoenzyme (*259*). In addition to glucose, the enzyme also catalyzes the oxidation of D-galactose, D- and L-xylose, and D-arabinose (*260*). Liver glucose dehydrogenase is strongly and competitively inhibited by glucose-6-phosphate or fructose-1,6-diphosphate. The inhibition constant (K_i) for the diphosphate is 6.2×10^{-5} M, and that for G-6-P is 2.5×10^{-6} M (259).

The physiological function of glucose dehydrogenase is obscure. Brink has suggested (222) that the oxidation of glucose to gluconic acid (catalyzed by this enzyme) is not a normal metabolic pathway of glucose. This is in line with the findings from isotopic studies by Stetten and Stetten (*261*), who have concluded that the direct oxidation of glucose catalyzed by the liver dehydrogenase contributes little, if any, gluconic acid to the body fluid.

Glucose dehydrogenase has also been found in yeast (*262*) and in extracts of *Candida reukaufii* and *Endomyces vernalis* (*263*). However, the latter uses methylene blue as the acceptor. Further characterization remains to be done.

In most systems the proposal of a lactone as the first intermediate in the glucose oxidation is not based on the direct study of the enzymatic reaction. However, a lactone-splitting enzyme has been studied, and its purification from yeast has been achieved with at least 36-fold concentration (*248*). This enzyme has been also found in bacteria, molds, and mammalian tissues. It requires divalent ions, such as Mg^{2+}, Mn^{2+} or Co^{2+} for activity. Only those lactones which possess the same configuration as D-glucono-δ-lactone are active. Among the compounds tested with the purified lactonase only D-glucono-δ-lactone and 6-phosphoglucono-δ-lactone show 100% hydrolysis in 5 minutes; lactobionic-δ-lactone, D-glucono-γ-lactone, and D-galacto-γ-lactone are much slower; whereas D-mannono-δ-lactone, D-xylono-γ-lactone, D-glycero-D-galactonoheptono-γ-lactone, D-saccharo-δ-, D-gulono-γ-, D-glucurono-γ-, D- and L-arabono-γ-, D-talono-γ-, L-galactono-γ-, D-glycero-D-idoheptono-γ-, and D-glycero-D-galactono-γ-lactones, lactide, and pantoyl lactone are inactive. The enzymatic hydrolysis follows the first-order kinetics in contrast to the nonenzymatic reaction of zero order (*248*).

B. MULTIPLE CATABOLIC PATHWAYS OF GLUCOSE IN *Acetobacter suboxydans*

Acetobacter suboxydans is the one of a few organisms which have been extensively studied and possess divergent pathways in the oxidation of glucose, as summarized in Fig. 11. This colorful microorganism was first isolated in 1924 by Kluyver and DeLeeuw (*264*). Prior to 1952, however, practically all biochemical reactions were studied in fermentation media or at most with resting cells. Consequently it was believed that this organism had only a very restricted oxidation capacity; the yields of sorbose from sorbitol were more than 85%, and there was nearly quantitative conversion of glycerol to dihydroxyacetone, or of glucose to gluconic acid. Reviews on earlier work have appeared (*265*, *266*). In 1952, two important techniques for the study of this microorganism were introduced. They are use of 2,4-dinitrophenol, toward which *A. suboxydans* shows unusual behavior, and the methods of cell-free preparations (*267–273*). Since then both methods have been extensively used in other laboratories (cf., for example, references *273–276*).

It has been found that *A. suboxydans* not only can extensively oxidize polyalcohols and sugars, but also possesses a multiple pathway in the oxidation of other substrates. Glucose, as mentioned (*144*, *277*), is catabolized by the pentose cycle, initially through the hexokinase reaction. All enzymes in this pathway exist in soluble (nonparticulate) extracts and have been described in preceding sections. On the other hand, the particulate fraction, which also contains most of the cytochromes in the organism, catalyzes the pyridine nucleotide-independent oxidation of glucose to δ-D-gluconolactone. An enzyme responsible for the hydrolysis of the lactone has also been demonstrated.

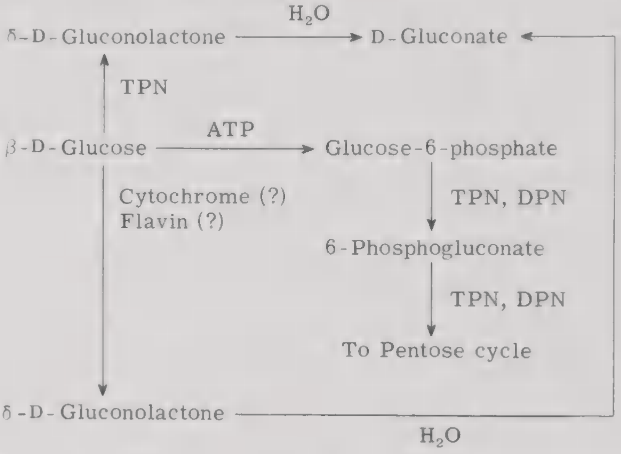


FIG. 11. Multiple catabolic pathways of glucose in *Acetobacter suboxydans.*

Another enzyme which catalyzes the oxidation of D-glucose to δ-D-gluconolactone is found in the soluble fraction. Among the sugars and sugar phosphates tested, only D-glucose and 2-deoxy-D-glucose are oxidized. Glucose-6-phosphate, glucose-1-phosphate, and 6-phosphogluconate are neither oxidizable nor inhibitory in contrast to the extremely potent inhibition that these phosphates display toward mammalian liver glucose dehydrogenase (*259*). It is also different from the liver dehydrogenase in the coenzyme requirement; either DPN or TPN can be used, whereas the *A. suboxydans* enzyme is absolutely TPN-specific.[*] The kinetic evidence suggests a mechanism of reaction as follows (*279*):

$$\begin{gathered} E + T \rightleftharpoons ET \\ E + G \rightleftharpoons EG \\ EG + T \rightleftharpoons EGT \\ ET + G \rightleftharpoons EGT \\ EGT \rightleftharpoons E + P + TH_2 \end{gathered}$$

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[*] In the preliminary paper (*278*) it was mistakenly written as DPN.

where E, G, P, T and TH_2 are for the dehydrogenase, glucose, gluconolactone (product), oxidized and reduced TPN, respectively. Thus it is similar to the reaction of yeast alcohol dehydrogenase (*280*) and creatine phosphokinase (*281*) rather than that of liver alcohol dehydrogenase extensively studied by Theorell and Chance (*282*, *283*). Using the technique similar to that developed by Dixon (*284*), the extrapolation of kinetic constants determined at different acidities suggests that both pK values of EGT and E are slightly higher than 7.0.

In comparing the properties of glucose oxidase, on the one hand, and glucose dehydrogenase as well as enzymes for the pentose cyclic oxidation of glucose on the other, several important but cryptic facts may be pointed out.

The particulate glucose oxidase can use 2,6-dichlorophenolindophenol to replace oxygen as electron acceptor. Triphenyltetrazolium chloride or methylene blue are practically inactive. This enzyme does not contain pyridine nucleotide. Its niacin content by the most rigid and sensitive tests gives a negative value. The particulate preparation, which has the full glucose oxidase activity, also contains flavin and practically all the cytochromes in the organism. Very recently Iwasaki (*285*) has confirmed our results in the oxidation behavior of glucose in this organism, but he apparently did not fully appreciate the concept of multiple catabolic pathways of glucose. Although our preparation catalyzes the decomposition of hydrogen peroxide, neither the rate of the oxidation nor the total amount of oxygen is changed by addition of ethanol, high concentrations of acetate or crystalline catalase (*286*). These results may point to water rather than hydrogen peroxide as the primary product. Moreover, under anaerobic conditions glucose reduces immediately all the cytochromes in the preparation, as sodium dithionate does. Although the behavior of flavin in the glucose oxidation catalyzed by the particulate preparation has not been extensively studied, these findings indicate that the mode of this oxidation differs from that of the mold oxidase. On the other hand, the pattern is very close to particulate succinic oxidase such as that in the Keilin-Hartree preparation. This organism cannot oxidize succinic acid. It may well be that glucose takes the place of succinic acid in relation to the occurrence of a "twin headed" (glucose and DPNH) respiratory chain, similarly to succinic and DPNH in mammals.

Another property that merits discussion is the effect of pH upon the enzymatic activity. The optimum pH value for glucose oxidase is 5.5. At acid pH ranges the reduced cytochrome spectra in the particulate preparation can hardly be demonstrated in aerobic conditions because the reduced cytochromes are rapidly oxidized by molecular oxygen. On the other hand, at pH 8.0, the reduced spectrum from glucose does not reach a maximum until after more than 15 minutes because the electron transfer from glucose to cytochrome is much slower than at pH 5.5, but the autoxidation of the reduced cytochrome is even slower. All these observations are in line with the fact that the oxidation by molecular oxygen in this organism shows optimal pH values of 5 to 6 (*267*); the pH optimum for growth is likewise 5.5 to 6 (*271*). In contrast, the pyridine nucleotide-dependent enzymes (both the nonparticulate glucose dehydrogenase and all the oxidative enzymes in the soluble extracts required for the pentose cycle), show maximal activities at much higher pH values than 7. In the region near pH 5.5, their actions are virtually nil. At the same time, the nonparticulate soluble extract has very limited ability to use molecular oxygen; in fact it contains practically no cytochromes. It seems that a concept of an intracellular pH gradient or compartmentation must be proposed in order to accommodate the observation. However, in resting-cell experiments at pH 6.0 when molecular oxygen is used as the final acceptor, the oxidation of glucose exhibits a diphasic curve. The initial rapid rate is evidently due to the first reaction in this formation of gluconate, and later the slow reaction is from further oxidation. These facts lead us to believe that in resting cells the pyridine nucleotide independent particulate glucose oxidase plays the predominant role in the oxidation of glucose. Thus in fermentation media, rapid aeration is needed for the maximal growth, and high yields of gluconic acid can be attained at pH 5.5. On either side of the optimal value, the gluconic acid formed is greatly decreased.

C. FURTHER OXIDATION OF GLUCONIC ACID

Studies on the further oxidation of D-gluconic acid have been almost exclusively made in microorganisms. The 2-keto acid is formed in good yield from not only D-gluconate but also other aldonic acids such as L-idonate and D-galactonate by *Pseudomonas* (*286a*, *287*) and by *Acetobacter* species (*155*, *156*, *275*, *276*, *288–290*). Kulhánek has generalized that those aldonic acids have the same configuration as gluconate in the first 3 carbon atoms are oxidizable at position 2 (*291*, *292*). However, Khesghi *et al.* (*293*) have found that D-gluconate is oxidized only to 5-ketogluconate in *Acetobacter suboxydans* whereas Claridge *et al.* have reported that both glucose and gluconate are oxidized to 2-ketogluconate (*294*, *295*). On the other hand, Kulka and Walker (*296*) have tested many species of *Acetobacter* and found that eight species can oxidize gluconate to both 2- and 5-ketogluconate. Although 2,5-diketogluconate is a product of gluconate oxidation (*297*, *298*), 5-ketogluconate is not the precursor of the diketo acid (*299*).

A more conclusive study has been reported by DeLey and co-workers (*274, 300*). They have isolated two soluble and one particulate enzyme from cell-free preparations of *A. suboxydans*. Both soluble enzymes are TPN-specific in catalyzing the oxidation of gluconate to 2-ketogluconate and 5-ketogluconate, respectively. They are named 2-ketogluconate reductase and 5-ketogluconate reductase (*300*). The particulate enzyme is possibly cytochrome linked and catalyzes the gluconate oxidation to 2-ketogluconate. 2-Ketogluconate reductase has been also partially purified from *Corynebacterium helvolum*, from yeast, *Debaryomyces hansenii*, and from the mold *Aspergillus nidulans* (*301*).

With extracts of *Aerobacter cloacae*, DeLey (*302, 303*) has found another soluble enzyme, 2-ketogluconokinase, which catalyzes the following reaction (*91*):

$$\text{2-Keto-D-gluconic acid} + \text{ATP} \xrightarrow{\text{Mg}^{++}} \text{2-Keto-6-phospho-D-gluconic acid} + \text{ADP}$$

The product upon hydrolysis yields 2-ketogluconic acid. The Ghent school in this connection has made an impressive survey on the metabolism of a great number of microorganisms and their ability to form 2-keto-6-phospho-D-gluconic acid by cell-free extracts (*304*). Table VIII is adapted from DeLey and Vandamme (*304*). As can be seen from the table, a great number of organisms can grow on 2-ketogluconate, but none tested contains constitutive 2-ketogluconokinase. Some doubt would thus be cast on the importance of the kinase in normal metabolism since it is only an inducible enzyme.

The literature in the field is quite diffuse although the scale of studies by a number of schools is impressive. To date a few hundred organisms have been scanned for gluconate metabolism. However, even the slightest indication of relationship between the biochemical pattern and the evolutionary scale cannot be traced out, nor can a general rationalization be gained in spite of the real existence of species specificity. These facts are true not only with respect to the activities just described, but also regarding other physiological reactions. One consequently would wonder whether the tactics of attacks now prevalent and the areas of reactions chosen are the most profitable or efficient to pursue the discipline of comparative biochemistry.

D. POLYOL DEHYDROGENASES

Investigations on the biochemical oxidation of polyols were begun as early as the middle of the nineteenth century. Pelouze (*305*) fermented the juice of mountain ash berries in open vessels and obtained an optically active, crystalline, unknown compound. However, it was not until 1896–1898 that Bertrand (*306*) was able to identify the product as

TABLE VIII

DECOMPOSITION OF 2-KETO-D-GLUCONATE BY VARIOUS BACTERIA, YEASTS, AND MOLDS

Organism[a]	Degree of growth[b]	Amount of 2-keto-gluconate decomposed (%)	O_2 uptake of washed cells[c]	Formation of 2-keto-D-gluconate-δ-phosphate by cell-free extract[d]
Pseudomonas aromatica	++++	75	Nt	+++
P. aeruginosa	+++	71	Nt	++
P. putida	+++	34	Nt	Nt
P. aromatica var. *quercitopyrogallica*	+++	73	Ind.	++
P. fluorescens (2)	+++	32–73	Ind.	+
P. ovalis	+++	40	Nt	+++
P. convexa	+++	75	Nt	Nt
P. viscosa	+++	77	Nt	Nt
Xanthomonas phaseoli	+	5	Nt	+
X. campestris	+	3	Nt	+
X. pruni	+	7	Nt	±
Agrobacterium tumefaciens	++++	73	Ind.	−
Corynebacterium simplex (2)	++++	40–69	Nt	−
C. helvolum	++++	73	Ind.	−
C. poinsettiae	+++	15	Nt	−
Flavobacterium aquatile	+++	17	0	−
Bacterium (Escherichia)				
B. coli I	+(++)	5–18	0	Nt
B. coli I	++++	90	Nt	Nt
Bacterium coli II (3)	++++	59–100	Nt	++
B. coli-anaerogenes	+	6	Nt	Nt
B. intermedium I (4)	++++	100	Ind.	+++
B. intermedium II	++++	100	Nt	Nt
Bacterium (Aerobacter)				
B. aerogenes I (8)	++++	100	Ind.	+++
B. aerogenes II	+		Nt	Nt
B. aerogenes II (2)	++++	100	Nt	Nt
B. cloacae (4)	++++	100	Ind.	+++
Paracolobactrum aerogenoides	++++	100	Ind.	+++
Bacterium (Klebsiella)				
B. pneumoniae (3)	+++	27–100	Nt	Nt
B. rhinoscleromatis	++	24	Nt	Nt
B. ozaenae	+++	65	Nt	Nt
Serratia plymuthica (2)	+++	69	Ind.	++
Proteus vulgaris	+	13	0	−
Erwinia carotovora	++	11	0	+
Bacillus subtilis	+	20	0	−
B. cereus var. *mycoides*	+	17	Nt	Nt

TABLE VIII (*Continued*)

Organism[a]	Degree of growth[b]	Amount of 2-keto-gluconate decomposed (%)	O_2 uptake of washed cells[c]	Formation of 2-keto-D-gluconate-δ-phosphate by cell-free extract[d]
B. megatherium (4)	++(+++)	26–95	Ind.	++
B. circulans	+	31	Nt	Nt
B. mesentericus	++	33	0	—
Schizosaccharomyces pombe (2)	++	0–21	Nt	Nt
Endomycopsis capsularis	+++	39	Nt	Nt
Saccharomyces delbrueckii	+++	14	0	Nt
Schwanniomyces occidentalis (2)	++++	64–91	Ind.	—
Debaryomyces hansenii	++++	74	Ind.	—
Hanseniaspora apiculata	+++	37	0	Nt
Lipomyces lipoferus	++++	81	Ind.	—
Torulopsis holmii	+++	13	0	Nt
Brettanomyces bruxellensis	+++	10	Nt	Nt
Candida albicans	++++	100	Ind.	—
Kloeckera apiculata (2)	++(+++)	18–40	0	Nt
Neurospora sitophila	+++	75	Nt	—
Nectria cinnabarina	++	100	Nt	Nt
Monascus ruber	+++	76	Nt	—
Aspergillus nidulans	+++	82	Nt	Nt
A. fumigatus	+++	89	Nt	—
A. flavus	+++	92	Nt	
A. niger	+++	88	Nt	Nt
Pencillium chrysogenum	+++	92	Nt	—
Pencillium sp.	+++	90	Nt	—
Pencillium sp.	+++	94	Nt	Nt
Cercospora beticola	++	74	Nt	Nt
Helminthosporium sativum	+++	92	Nt	Nt
Monilia brunnea	+	27	Nt	Nt
Phoma betae	+++	82	Nt	—
Dematium pullulans	+	16	Nt	Nt

[a] The number in parentheses after the names of organisms gives the number of strains tested; if the name is not followed by a number in parentheses, only one strain was tested.

[b] Degree of growth: ++++, excellent; +++, good; ++, moderate; +, weak.

[c] Oxygen uptake of washed cells: 0, no uptake; Nt, not tested; Ind., only after induction period.

[d] Intensity of spot on paper chromatograms of 2-keto-D-gluconate-6-phosphate quinoxaline, after 3-hour enzyme treatment (= activity of cell-free extracts): +++, very intense, nearly complete phosphorylation; ++, intense, excellent phosphorylation; +, weak phosphorylation, ± very weak phosphorylation but with a definite enzyme activity; —, no enzyme activity detectable after 3 hours.

sorbose. Bertrand also proved Pelouze's fermentation to be a process of bacterial oxidation of sorbitol that occurred in the berry juice. Bertrand further isolated the "sorbose bacterium" or *Acetobacter xylinum* (*307*). He then tested a number of polyols and formulated a general rule about their oxidizability. Later the rule was supplemented by Hudson *et al.* (*308*), so that it is known as the Bertrand-Hudson rule. The generalization is that *Acetobacter* can oxidize the hydroxy group at the position next to the primary alcohol of only those polyols that possess the two *cis-vicinal* secondary hydroxyls adjacent to a primary alcohol group. In addition to Bertrand's pioneer work, subsequent investigations by Kluyver, Hudson, Fulmer, Underkofler, Edson, Shaw, Reichstein, Richtmyer, and others have confirmed the rule. Table IX, which is based on Fulmer

TABLE IX

OXIDATION OF POLYOLS BY *Acetobacter* SPECIES

No.	Alcohol	Product	Representative species and references
1	(CH_2OH CH_3 Ethanol	$COOH$ CH_3 Acetic acid	*A. suboxydans* (*309*))[a]
2	CH_2OH CH_2SH 2-Mercaptoethanol	$COOH$ CH_2SH Thioglycolic acid (predicted)	*A. suboxydans* (*310*)
3	CH_2OH $HCOH$ CH_2OH Glycerol	CH_2OH CO CH_2OH Dihydroxyacetone	*A. suboxydans* (*307, 311*)
4	CH_2OH $HCOH$ CH_3 Propylene glycol	Oxidized	*A. suboxydans* (*310*)
5	CH_2SH $HCOH$ CH_2OH 1-Thio-2,3-propanediol	CH_2SH CO CH_2OH 1-Thio-3-hydroxy-propanone-2 (predicted)	*A. suboxydans* (*310*)

[a] Not a polyol.

TABLE IX (*Continued*)

No.	Alcohol	Product	Representative species and references
6	CH_2OH HOCH HOCH CH_2OH *meso*-Erythritol	CH_2OH CO HOCH CH_2OH L-Erythrulose	*A. suboxydans* (*312, 313, 314*)
7	CH_2OH HCOH HCH CH_2OH 1,2,4-Butanetriol	Oxidized	*A. suboxydans* (*310*)
8	CH_3 HOCH HOCH CH_3 *meso*-2,3-Butanediol	CH_3 HOCH CO CH_3 L(+)-Acetyl methyl carbinol	*A. suboxydans* (*315, 316*) *A. xylinum* (*317*) *A. aceti* (*317*)
9	CH_3 HCOH HOCH CH_3 L(+)-2,3-Butanediol	Not oxidized	*A. suboxydans* (*316*) *A. xylinum* (*317*) *A. aceti* (*317*)
10	CH_3 HOCH HCOH CH_3 D(−)-2,3-Butanediol	CH_3 CO HCOH CH_3 D(−)-Acetyl methyl carbinol	*A. suboxydans* (*316*) *A. xylinum* (*317*) *A. aceti* (*317*)
11	CH_2OH CH ‖ CH CH_2OH 2-Butene-1,4-diol	Oxidized	*A. suboxydans* (*310*)
12	CH_3 COH ‖ COH CH_3 2,3-Butenediol	Oxidized	*A. suboxydans* (*310*)

TABLE IX (*Continued*)

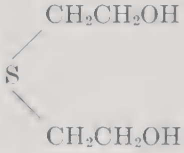
No.	Alcohol	Product	Representative species and references
13	CH_2OH C ||| C CH_2OH 2-Butyne-1,4-diol	Doubtful	*A. suboxydans* (*310*)
14	CH_2OH CH_3CNO_2 CH_2OH 2-Methyl-2-nitro-1,3-propanediol	Doubtful	*A. suboxydans* (*318*)
15	 CH_2CH_2OH S CH_2CH_2OH Thiodiethylene glycol	Oxidized	*A. suboxydans* (*310*)
16	CH_2OH HOCH HOCH HOCH CH_2OH *meso*-Adonitol (*meso*-Ribitol)	CH_2OH CO HOCH HOCH CH_2OH L-Adonose (L-Ribulose)	*A. suboxydans* (*319*)
17	CH_2OH HCOH HOCH HOCH CH_2OH L-Arabitol	Not oxidized	*A. suboxydans* (*308*)
18	CH_2OH HOCH HCOH HCOH CH_2OH D-Arabitol	CH_2OH CO HOCH HCOH CH_2OH D-Xylulose	*A. suboxydans* (*308*)
19	CH_2OH HCOH HOCH HCOH CH_2OH *meso*-Xylitol	Not oxidized	*A. suboxydans* *A. xylinum* (*308, 320*)

TABLE IX (*Continued*)

No.	Alcohol	Product	Representative species and references
20	CH_3 HOCH HCOH HCOH CH_2OH D-Lyxomethylitol (1-Deoxy-D-arabitol)	Oxidized	*A. suboxydans* (*321*)
21	CH_3 HCOH HOCH HOCH CH_2OH L-Lyxomethylitol (1-Deoxy-L-arabitol)	Oxidized	*A. suboxydans* (*321*)
22	CH_2OH HCH HOCH HCH CH_3 1,3-Pentanediol	Oxidized	*A. suboxydans* (*318*)
23	CH_2OH HCH HCH HCH CH_2OH 1,5-Pentanediol	Oxidized	*A. suboxydans* (*318*)
24	CH_2OH HOCH HOCH HCOH HCOH CH_2OH D-Mannitol	CH_2OH CO HOCH HCOH HCOH CH_2OH D-Fructose	*A. suboxydans* *A. xylinum* (*319*, *322*)
25	CH_2OH HCOH HOCH HCOH HCOH CH_2OH D-Sorbitol (D-Glucitol)	CH_2OH CO HOCH HCOH HOCH CH_2OH L-Sorbose[b]	*A. suboxydans* (*319*) *A. xylinum* (*323*)

[b] D-Fructose is also formed; see text.

TABLE IX (*Continued*)

No.	Alcohol	Product	Representative species and references
26	CH_2OH HCOH HOCH HOCH HCOH CH_2OH *meso*-Dulcitol	Not oxidized	*A. suboxydans* (*308, 324*)
27	CH_2OH HOCH HCOH HOCH HCOH CH_2OH D-Iditol	Not oxidized	*A. xylinum* (*306*)
28	CH_2OH HOCH HOCH HOCH HCOH CH_2OH D-Talitol	CH_2OH CO HOCH HOCH HCOH CH_2OH D-Tagatose (predicted)	*Acetobacter* ? (quoted in *325*)
29	CH_2OH HOCH HOCH HOCH HOCH CH_2OH *meso*-Allitol	CH_2OH CO HOCH HOCH HOCH CH_2OH L-Allulose	*A. suboxydans* *A. xylinum* (*326*)
30	CH_3 HCOH HCOH HOCH HOCH CH_2OH L-Rhamnitol	Not oxidized	*A. suboxydans* (*308, 321, 324*) *A. xylinum* (*327*)
31	CH_3 HOCH HOCH HCOH HCOH CH_2OH D-Rhamnitol	Oxidized	*A. suboxydans* (*321*)

TABLE IX (*Continued*)

No.	Alcohol	Product	Representative species and references
32	CH_2OH HCH HOCH HCOH HCOH CH_2OH 2-Deoxy-D-sorbitol	CH_2OH CO HOCH HCOH HCH CH_2OH 5-Deoxy-L-sorbose	*A. suboxydans* (*325*)
33	CH_2OH HOCH HCOH HCOH HOCH CH_3 6-Deoxy-L-galactitol (L-Fucitol)	CH_2OH HOCH HCOH CO HOCH CH_3 L-Fuco-4-ketose	*A. suboxydans* (*308, 328*)
34	CH_2OH HCOH HOCH HOCH HCOH CH_3 D-Fucitol (Rhodeitol)	Not oxidized	*A. xylinum* (*327*)
35	CH_2OH HCOH HCOH HCOH HCOH CH_3 D-Allomethylitol	Oxidized	*A. suboxydans* (*321*)
36	CH_2OH HOCH HCOH HOCH HOCH CH_3 L-Glucomethylitol	Doubtful	*A. suboxydans* (*321*)
37	CH_2OH HOCH HOCH HCOH HOCH CH_3 L-Gulomethylitol	Oxidized	*A. suboxydans* (*321*)

TABLE IX (*Continued*)

No.	Alcohol	Product	Representative species and References
38	CH_2OH HCOH HCOH HOCH HCOH CH_3 D-Gulomethylitol	Oxidized	*A. suboxydans* (*321*)
39	CH_2OH HOCH HCOH HOCH HCOH CH_3 D-Idomethylitol	Not oxidized	*A. suboxydans* (*321*)
40	CH_2OH HCH HCH HCH HCH CH_2OH *n*-Hexane-1,6-diol	Oxidized	*A. suboxydans* (*310*)
41	CH_3 HCOH HCH HCH HCOH CH_3 2,5-Hexanediol	Oxidized	*A. suboxydans* (*318*)
42	CH_2OH HCOH HCOH HOCH HCOH HCOH CH_2OH *meso-gluco-gulo*-Heptitol (α-Glucoheptitol)	CH_2OH CO HOCH HCOH HOCH HOCH CH_2OH L-*gluco*-L-*sorbo*-Heptose (L-Eseucoheptulose)	*A. suboxydans* (*308*) *A. xylinum* (*329*)

TABLE IX (*Continued*)

No.	Alcohol	Product	Representative species and References
43	CH_2OH HOCH HOCH HOCH HCOH HCOH CH_2OH D-*manno*-D-*talo*-Heptitol (D-Volemitol, α-Sedoheptitol)	(**A**) CH_2OH CO_2 HOCH HOCH HCOH HCOH CH_2OH D-*manno*-D-*tagato*-Heptose (D-Mannoheptulose)	*A. suboxydans* (*350*) *A. xylinum* (*306*)
		(**B**) CH_2OH CO HOCH HCOH HCOH HCOH CH_2OH D-*altro*-D-*fructo*-Heptose (D-Altroheptulose)	
44	CH_2OH HOCH HCOH HOCH HCOH HCOH CH_2OH D-*gluco*-D-*ido*-Heptitol (D-β-Glucoheptitol)	CH_2OH CO HOCH HCOH HOCH HCOH CH_2OH D-*ido*-L-*sorbo*-Heptose (predicted)	*A. xylinum* (*325*)
45	CH_2OH HOCH HCOH HOCH HOCH HCOH CH_2OH D-*gala*-L-*gluco*-Heptitol (D-β-Galaheptitol)	Not oxidized	*A. suboxydans* (*308*)

TABLE IX (*Continued*)

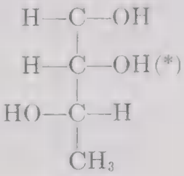
No.	Alcohol	Product	Representative species and References
46	CH_2OH HOCH HCOH HCOH HOCH HOCH CH_2OH L-Perseitol	Not oxidized	*A. suboxydans* (*308*)
47	CH_2OH HCOH HOCH HOCH HCOH HCOH CH_2OH D-*manno*-D-*gala*-Heptitol (D-Perseitol)	CH_2OH CO HOCH HCOH HCOH HOCH CH_2OH L-*gala*-D-*fructo*-Heptose (L-Perseulose, L-Galaheptulose)	*A. suboxydans* (*308, 331*) *A. xylinum* (*332*)
48	CH_2OH HOCH HCOH HCOH HOCH HOCH CH_3 L-*manno*-L-*gala*-7-Deoxyheptitol (α-Rhamnohexitol)	Not oxidized	*A. xylinum* (*327*)
49	CH_2OH HCOH HCOH HCOH HOCH HOCH CH_3 L-*manno*-L-*talo*-7-Deoxyheptitol (β-Rhamnohexitol)	Not oxidized	*A. xylinum* (*327*)

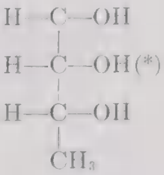
TABLE IX (*Continued*)

No.	Alcohol	Product	Representative species and references
50	CH_2OH HCOH HCOH HCOH HOCH HCOH HCOH CH_2OH D-*gluco*-L-*talo*-Octitol (D-α,α-Glucooctitol)	CH_2OH CO HOCH HCOH HOCH HOCH HOCH CH_2OH L-*altro*-L-*sorbo*-Octose (predicted)	*A. suboxydans* (*308*)
51	CH_2OH HOCH HCOH HCOH HOCH HOCH HCOH CH_2OH D-*gala*-L-*gala*-Octitol (D-α,α-Galactitol)	Not oxidized	*A. suboxydans* (*308*)

and Underkofler's work, summarizes the oxidation of polyols by *Acetobacter* sp.

It can be seen from the table that the rule can be applied not only to *A. xylinum*, which was the bacterium originally studied by Bertrand, but also to other species. There is no exception (see below). However, Hudson *et al.* (*330*) have found that L-fucitol, which is a deoxypolyol and does not fulfill the requirement of the rule, is oxidized to L-fuco-4-ketose. D-Fucitol is not attacked. A slight modification of the general rule has therefore been advanced. According to Hudson (*330*), only those ω-deoxy polyols with the configuration (III) or (IV)


(III)


(IV)

are oxidizable by *Acetobacter* species at the position marked with asterisk (*). This is reasoned from the fact that the CH_3CHOH group acts as if it were a CH_2OH group. One of the interesting tests for the Bertrand-Hudson rule is the oxidation of D-volemitol. The polyol contains two positions, namely C-2 and C-6, labile to oxidation. Although the diketo derivative has not been found, two ketoses, D-mannoheptulose and sedoheptulose, have been isolated in approximately equal yields from the action of *A. suboxydans* on the polyol (*330*).

After critical examination* of the literature, McCorkindale and Edson (*333*) have further generalized the Bertrand-Hudson rule. They have advanced a unified theory that the enzyme responsible for reactions prescribed by the rule is really due to a DPN linked, nonparticulate soluble catalyst (*330, 334*) and have named it D-mannitol dehydrogenase. However, this hypothesis has been challenged by the simultaneous discoveries by Edson's group (*335*) and by this laboratory (*310, 318, 322*) on another pathway in the oxidation of sorbitol. The formation of L-sorbose from D-sorbitol catalyzed by *Acetobacter* has been known since the first experiment of Bertrand. In fact, this reaction is the only commercial source for this keto sugar. In 1956, it was found that the oxidation of sorbitol by *A. suboxydans* yields not only L-sorbose, but also D-fructose (*318, 322, 335, 336*). In a cell-free system, the pathway is determined by the kind of pyridine nucleotide present; the reaction that forms sorbose is TPN dependent whereas the other requires DPN. Here the "D-mannitol dehydrogenase" of McCorkindale and Edson as a DPN-linked soluble enzyme cannot be applied.

This dual pathway of sorbitol oxidation is further characterized by the partial separation of the two enzymes. The products are also identified by conventional means, such as melting points, paper chromatography, polarimetry, and the crystalline form of the osazones. If these observations are correct, then the oxidation of sorbitol to fructose by *A. suboxydans* is the first real exception to the well-known Bertrand rule. A similar dual pathway has been found in *Candida utilis* in the oxidation of sorbitol (*335*) to fructose and sorbose. This organism also resembles *A. suboxydans* in the oxidation of mannitol to fructose, in the oxidation of xylitol to xylulose, and in the failure to attack dulcitol.

Mention must be made that the oxidation of sorbitol in *A. suboxydans* can be catalyzed by another enzyme (cf. Fig. 12). This enzyme (*335, 337*) differs from the two previously described in that it occurs in the particulate fraction, shows a pH optimum at 6, and is pyridine nucleotide independent. The product is presumably L-sorbose.

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* See Hudson (*332a*) and Carr (*332b*) for the chemistry of, and early studies on, metabolism of polyols.

The second general class of polyol dehydrogenases proposed by Edson is galactitol dehydrogenase (*333, 338–340*). This enzyme occurs in several microorganisms and has been studied in considerable detail in a fluorescent species of *Pseudomonas*. It catalyzes the oxidation of dulcitol to D-tagatose and sorbitol to D-fructose. DPN is required as the coenzyme. Specificity tests show that those polyols containing an L-threo configuration adjacent to a primary alcohol group are attacked. Experimentally these include the oxidation of L-iditol to L-sorbose, of L-arabitol to L-ribulose, and of xylitol to L-xylulose.

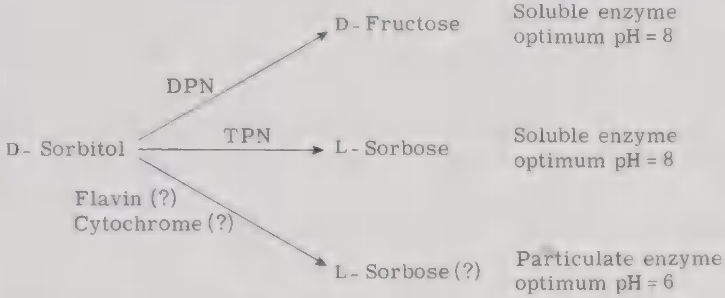


FIG. 12. Multiple oxidation of sorbitol by *Acetobacter suboxydans*.

The oxidation of sorbitol to fructose has been well demonstrated in animal tissues. Blakley (*341*) has isolated a dehydrogenase from rat liver homogenates that catalyzes the following reaction:

$$\text{D-Sorbitol} + \text{DPN}^+ \rightleftarrows \text{D-Fructose} + \text{DPNH} + \text{H}^+$$

TPN is not active (*341, 342*). The reaction is reversible; the equilibrium constant has been determined:

$$K = \frac{(\text{Fructose})(\text{DPNH})}{(\text{Sorbitol})(\text{DPN})} = 0.24 \text{ at } 20^\circ \text{ and pH } 8.0 \ (341)$$

$$K = \frac{(\text{Fructose})(\text{DPNH})(\text{H}^+)}{(\text{Sorbitol})(\text{DPN}^+)} = 2 \times 10^{-9}\ M \text{ at } 25^\circ \ (342)$$

Although fructose is the only oxidation product, it is interesting to note that the reverse reaction can also take place with L-sorbose instead of fructose at only a slightly slower rate.

This enzyme also catalyzes the following reactions:

Ribitol ⇆ D-Ribulose
Xylitol ⇆ D-Xylulose
Allitol ⇆ D-Allulose
L-Iditol ⇆ L-Sorbose
L-*gulo*-D-*gala*-Heptitol ⇆ L-*gala*-Heptulose (perseulose)
L-*gulo*-D-*talo*-Heptitol ⇆ L-*altro*-Heptulose (sedoheptulose)

However, *meso*-erythritol, D- or L-arabitol, galactitol (dulcitol), L-glucitol (D-gulitol), 6-deoxy-L-gulitol, D-iditol, D-mannitol, D-talitol (D-altritol), and D-*manno*-D-*gala*-heptitol are not oxidized. It is believed that these reactions are catalyzed by one enzyme. L-Iditol dehydrogenase is the generic name given by Edson (*333, 340*), but the name sorbitol dehydrogenase is perhaps more generally known.

This enzyme has been also found in sexual accessory organs (*342, 343*). Since it shows a group specificity and since the equilibrium favors the formation of the polyol, the enzyme has also been named ketose reductase (*342*). Thus the names L-iditol dehydrogenase, sorbitol dehydrogenase, and ketose reductase may actually refer to one enzyme.

Sorbitol dehydrogenase has recently been isolated from semen (*344*). Mammalian semen is an interesting body fluid which contains a high concentration of fructose, but no glucose (*345*). Moreover, sorbitol has also been found to exist in high content (*346*). Properties of the dehydrogenase isolated from spermatozoa resemble those from liver (*333, 341, 347*) and from the seminal vesicle (*342, 343*). A comparison of their specificities is listed in Table X.

TABLE X

SPECIFICITY OF SORBITOL DEHYDROGENASE IN THE OXIDATION OF POLYOLS

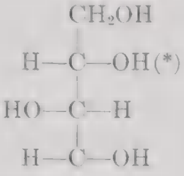
Polyol	Ram spermatozoa (*348*)	Guinea pig seminal vesicle (*342, 343*)	Mammalian liver (*333*)
D-Sorbitol	100	100	100
L-Iditol	90	101	96
β-Sedoheptitol	—[a]	—	87
D-Xylitol	79	51	85
D-Ribitol	36	76	49
L-*gulo*-D-*gala*-Heptitol	—	—	52
Allitol	—	—	45
D-Mannitol	17	5	0
D-Arabitol	4	0	0
meso-Inositol	0	0	0
meso-Erythritol	0	—	0

[a] Dash means not tested.

In the reversible reaction, the semen enzyme catalyzes the reduction of fructose and, at a slower rate, of L-sorbose. The latter presumably yields L-iditol. No reduction is obtained with D-ribose, 2-deoxy-D-glucose, D-mannose, or D-glucose. Thus the enzymes isolated from animal origin—semen (*345*), mammalian liver (*341, 342, 347*), and seminal vesicle (*343*)—show the same specificity range. The slight activities on

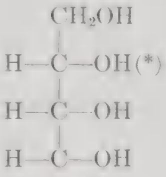
D-mannitol and D-arabitol by the semen dehydrogenase may be due to impurities in the polyols used. These enzymes thus are most probably identical.

Based on these specificity tests with the liver enzyme, Edson (*333*, *340*) has generalized that L-iditol dehydrogenase can oxidize only those compounds at the positions marked with an asterisk (*) with the configurations (V) or (VI).



$$\begin{array}{c} CH_2OH \\ | \\ H{-}C{-}OH(^*) \\ | \\ HO{-}C{-}H \\ | \\ H{-}C{-}OH \end{array}$$

(V)



$$\begin{array}{c} CH_2OH \\ | \\ H{-}C{-}OH(^*) \\ | \\ H{-}C{-}OH \\ | \\ H{-}C{-}OH \end{array}$$

(VI)

The oxidation of polyols to sugar as such does not yield useful energy. The further oxidation of the reduced pyridine nucleotide via the respiratory chain system is exergonic. Nevertheless as far as the reaction in the animal system is concerned, its significance as a useful energy-yielding process is doubtful. It is obvious that the oxidation of sorbitol to fructose by intact spermatozoa in semen requires DPN whereas the reverse process depends on the availability of DPNH. The slow rate at which fructose is reduced by spermatozoa may be due to the limited supply of DPNH which becomes available slowly but continuously during the oxidation of triose phosphate in the normal course of sperm fructolysis. On the other hand, the equilibrium of the sorbitol oxidation favors its formation from fructose, which is abundant in semen. At the same time, the Michaelis constant of the enzyme for sorbitol is rather high.

In intact spermatozoa, however, the steady state of the reversible oxidation of sorbitol to fructose depends upon the actual ratio of DPN and DPNH in the sperm cells. This ratio in turn is related to the rate at which the various DPN-catalyzed metabolic reactions (glycolysis, for example) proceed in the semen, and it is also bound up with the state of the terminal oxidation which involves the cytochromes and availability of molecular oxygen. Among the oxidizable substances in semen, fructose and sorbitol occur in remarkably large but variable concentrations. It is conceivable that the respective levels of fructose and sorbitol in semen can affect the ratio of DPN and DPNH in spermatozoa, which is under the control of sorbitol dehydrogenase. Therefore, the enzyme may serve as a metabolic regulator rather than play the role of a conventional oxidative enzyme (*348*). This reasoning is in line with the mechanism of glucose-fructose conversion first discovered by Hers (*349*).

An enzyme that has been isolated from seminal vesicles catalyzes the following reaction:

$$\text{Glucose} + \text{TPNH} + \text{H}^+ \rightleftharpoons \text{Sorbitol} + \text{TPN}^+$$

Since the equilibrium position favors the formation of sorbitol the name aldose reductase has been proposed (*350, 351*). This enzyme also occurs in the fetal blood of ungulates, placentas of certain mammals, fetal liver, and other tissues. In addition to glucose, such compounds as D-glucosone, D-glucuronolactone, and D-glyceraldehyde are also active. This aldose reductase reaction coupled with the reaction of sorbitol dehydrogenase (ketose reductase) explains the glucose-fructose conversion without involvement of any phosphorylated sugar intermediates as follows:

Aldose reductase: $\text{Glucose} + \text{TPNH} + \text{H}^+ \rightleftharpoons \text{Sorbitol} + \text{TPN}^+$
Ketose reductase: (Sorbitol dehydrogenase) $\text{Sorbitol} + \text{DPN}^+ \rightleftharpoons \text{Fructose} + \text{DPNH} + \text{H}^+$

Over-all: $\text{Glucose} + \text{TPNH} + \text{DPN}^+ \rightleftharpoons \text{Fructose} + \text{TPN}^+ + \text{DPNH}$

Hers (*351*) has used this scheme to explain the formation of seminal fructose under physiological conditions since the tissue contains both enzymes. The discovery (*346*) of sorbitol in mammalian semen certainly enhances the argument of the proposed mechanism of these enzymes as metabolic regulators (*348*). At the same time it explains the apparent irreversibility of glucose → sorbitol → fructose transformation due to a high ratio of $(\text{TPNH})(\text{DPN}^+)/(\text{TPN}^+)(\text{DPNH})$ (*352*). This transformation has also been shown in the normal and diabetic human (*353*), and in silkworms (*354*), during hypothermia in man.

Nearly a hundred years ago Lord Kelvin said: "When you can measure what you are speaking about and express it in numbers, you know something about it, and when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meager and unsatisfactory kind." But a hundred years later, so far as the areas just reviewed are concerned, we may still be a long way from reaching a satisfactory kind of knowledge. Although progress is being made, comparative biochemistry and the metabolic pathways have not yet reached the stage that we can say: "*Natura enim simplex est!*"

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CHAPTER 10

Terpenoids: Structure and Distribution *

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I. Scheme of Terpenoid Structure 503
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E. Triterpenes 510
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I. Scheme of Terpenoid Structure

The parent compounds of the terpenes and terpenoids are multiples of an unsaturated isopentane unit (Table I). Up to diterpenes, the carbon skeletons are lined up one next to the other, but the synthesis of tri- and tetraterpenes is achieved through the dimerization of precursors half their size. Mono- and sesquiterpenes often occur as hydrocarbons. By oxidation, these can be converted to alcohols, oxides, ketones, aldehydes, acids, and lactones. Triterpene compounds are known almost exclusively in the form of their oxygen derivatives. In certain cases the original terpenoid is hydrogenated (phytol) or dehydrogenated (carotenoids). Still other alterations in the original terpenoids lead, by way of oxidative degradation, to numerous steroid derivatives (bile acids, sex hormones, etc.); and by reaction with other compounds, to mixed terpenoids (alkaloids of the delphinium, aconitum, solanum, and veratrum groups, vitamins E and K, certain coumarins, and the like).

As one can see from Scheme I and Chapter 11 on metabolism of terpenoids, the isopentane units ("active isoprene") are always aligned according to (I*a*). The "irregularly" constructed coupling (I*b*) which is found in some terpenoids, can be formed only through secondary changes. These play a part in certain mono-, sesqui-, and diterpenes. By

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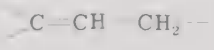
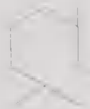
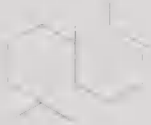
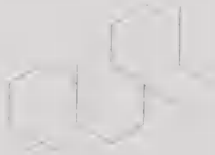
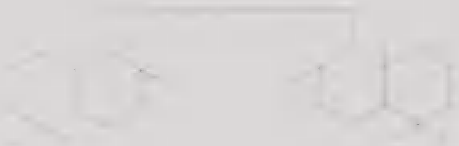
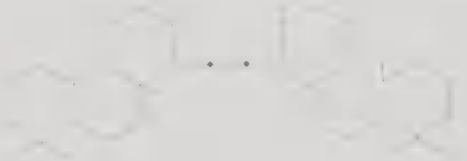
* Translated from the German by Dr. Herbert S. Heineman, University of Pittsburgh School of Medicine.

TABLE I

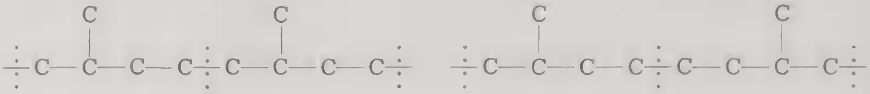
Scheme of Terpenoid Structure

Terpene class	Empirical formula	Carbon skeleton	Examples
Hemiterpene compounds	C_5H_8-residue = 1 x C_5	 $C=CH-CH_2-$	Hemiterpene compounds (Table II)
Monoterpenes	$C_{10}H_{16}$ = 2 x C_5		Terpene compounds (Tables IV - VI)
Sesquiterpenes	$C_{15}H_{24}$ = 3 x C_5		Sesquiterpenes and related compounds (Tables VII - IX)
Diterpenes	$C_{20}H_{32}$ = 4 x C_5		Diterpene compounds: resin acids; hydrogenated: phytol (in chlorophyll, vitamins E and K); dehydrogenated: vitamin A (Table X)

Terpene class	Empirical formula	Carbon skeleton	Examples
Triterpenes	$C_{30}H_{48} = 6 \times C_5$ $= 2 \times C_{15}$		Triterpenes (Tables XI, XII) Steroids: sterols, bile acids, sex and adrenal hormones, vitamin D, cardiac glycosides (digitalis, etc.) Alkaloids: strophanthin, solanidine, veratrine, toad poisons
Tetraterpenes	$C_{40}H_{64} = 8 \times C_5$ $= 2 \times C_{20}$		Known in dehydrogenated form as carotenoids
Polyprenes	$(C_5H_8)_n$	 Caoutchouc (= *cis* -1, 4-polyprene) Guttapercha and balata (= *trans* -1, 4-polyprene)	$n > 100,000$, Types of caoutchouc

SCHEME I

Regular coupling of isoprene units *Irregular coupling*

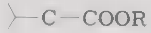


(tail-to-head)
(a)

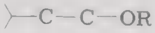
(tail-to-tail)
(b)

TABLE II

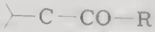
Hemiterpene Compounds (5, 6)

(II)

$\rangle\!-C-COOR$

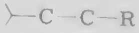
Isovaleric acid
ββ-Dimethylacrylic acid
Acacipetalin
Penicillins
Samidin
Athamantin
Pyrethrins

(III)

$\rangle\!-C-C-OR$

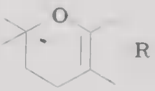
Isoimperatorin
Oxypeucedanin
Osthruthol
Imperatorin
Phellopterin
Byakangelicin
Evoxine

(IV)

$\rangle\!-C-CO-R$

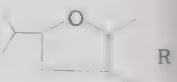
Geigerin
Humulone

(V)

$\rangle\!-C-C-R$

Peucenin
Auroptene
Osthole
Toddadolactone
Icariin
Lapachol
Flavoglaucin
Lomatiol
Meranzin
Suberosin
Psoralidin
Osagin
Pomiferin
Auroglaucin

(VI)


Evodion
Lapachenol
Seselin
Braylin
Xanthyletin
Luvangetin
Osajin
Pomiferin
Deguelin
Toxicarol
Tephrosin
Jacareubin
Rottlerin
Acronylin
Medicosmine
Mundulone
Acronidine
Flindersin

(VII)


Marmesin
Nodakenin
Visamminol
Rotenone
Oreoselone
Peucedanin
Euparin
Oroselone
Xanthoxyletin
Athamantin

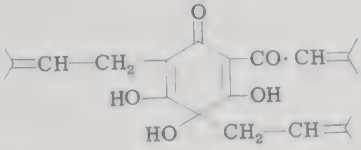
means of different types of cyclization, they lead to a number of irregularly constructed triterpenes.

II. Structure and Distribution of Terpenoids

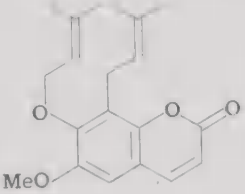
So that the structure (Tables II–XII) and distribution (Tables XIII–XVI) of terpenoids might be discussed succinctly, steroids and carotenoids have been left out of consideration. A tabular form of presentation seems to be the most convenient.* Citation of the literature has posed a great problem, since lack of space has necessitated a minimal allotment for references. For this reason, reference has been made in the table to standard works, such as those of Simonsen (*1*), Guenther (*2*), Gildemeister (*3*), de Mayo (*4*), and Karrer (*5*). Good reviews of individual topics are afforded by the following authors: Aneja and coworkers (*6*), hemiterpenes; Haagen-Smit (*7, 8*), lower terpenes, sesquiterpenes, and diterpenes; Jeger (*9*), triterpenes; Jones and Halsall (*10*), tetracyclic triterpenes; Steiner and Holtzem (*11*), triterpenes; and Tsutsui and Tsutsui (*12*), diterpenes.

A. Hemiterpene Compounds

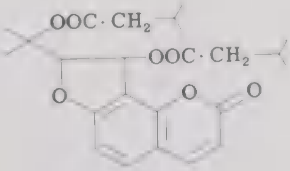
The C_5 unit of terpene compounds is an intermediary metabolite which, as such, does not accumulate in the plant. There are, however, a large number of natural substances in which an isoprenyl residue is bound in different ways (Table II, Types II–VII). In most cases ben-



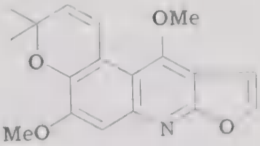
Humulone
(VIII)



Brayleyanin
(IX)



Athamantin
(X)



Acronidine
(XI)

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* Tables IV to XVI appear on pp. 511–586.

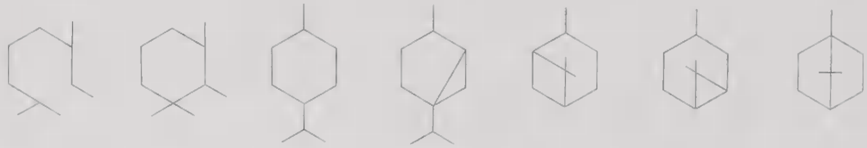
zene, naphthalene, quinoline, and benzopyrone derivatives are involved; these are discussed elsewhere. Here a few examples will be presented to illustrate the diverse linkages into which the hemiterpene residue may enter (VIII–XI). As will be discussed in greater detail later, the group of hemiterpenes has additional importance in that many natural furans are derived biogenetically from hemiterpenes (*6*).

B. TERPENES (*1*–*5*, *7*)

Terpenes and compounds derived therefrom basically contain one of the carbon skeletons shown in Table III (XII–XVIII). Besides those terpenes with regular structure (Table IV) (XIX–CXXV), there are those that, as a result of secondary transformations, show irregular coupling of the isopentenyl units (Table V) (CXXVI–CLXIII); through dehydrogenation, have been converted to aromatic compounds; or, through oxidative degradation, have lost carbon atoms (Table VI) (CLXIV–CLXXV).

TABLE III

Parent Compounds of Terpenes



(XII) (XIII) (XIV) (XV) (XVI) (XVII) (XVIII)

Geraniol frequently occurs in coumarins and other compounds in ether form [bergamotin, 8-geranylhydroxypsoralen, auraptene, collinin, mycelianamide, 7-methoxy-5-geranylhydroxycoumarin (*5*, *6*)] or in substituted form [chlorophorin, ostruthin (*5*)]. Ethers of geraniol hydrate [marmin (see reference *5*, p. 535)] and thymolhydroquinone (CLXI–CLXIII) are also known. In cannabidiol, limonene is a substituent on a benzene nucleus (reference *5*, p. 98). Most of the terpenes are constituents of essential oils. Other terpene compounds are of considerable biologic interest. Thus, 4-hydroxy-β-cyclocitral (XLV) in the green alga *Chlamydomonas eugametos* is the active agent which determines the male sex. Mesogentiogenin (XXXVIII) is the aglycon of gentiopicrin and iridomyrmecin (LXXX), a substance produced by certain ants as a weapon which is insecticidal, fungicidal, and bactericidal. Certain terpene compounds, notably the thujaplicins (CXLVI–CL), are the basis of the durability of woods.

C. Sesquiterpenes

The regularly constructed sesquiterpenes can be divided into aliphatic and cyclic (Tables VII and VIII). The most important representative of the former group is farnesol, which, bound to coumarin derivatives, also occurs as umbelliprenin (reference 5, p. 535) and ammoresinol (reference 5, p. 547). Only a few of the cyclic sesquiterpenes belong to the bisabolene, selinene, and iresin types (CLXXVI, CLXXVIII, CLXXIX, respectively), and the majority possess the carbon skeleton of cadinene (CLXXVII) or of an azulene. These basic skeletons are easily obtained through dehydrogenation. Thus, sesquiterpenes of the type (CLXXVII) yield cadalene (CLXXXI); those of the type selinene (CLXXVIII) yield eudalene (CLXXXII); and those of the type iresin (CLXXIX) yield 1,5-dimethylnaphthalene (CLXXXIII). Azulenoid sesquiterpenes can be dehydrogenated to blue or violet azulenes. Thus, compounds of the guaiol type (CLXXX) are converted to guaiazulene. If, on the other hand, the compound contains, on the isopropyl group, a terminal carboxyl or lactone, then chamazulene (CLXXXVI) is formed. In certain cases, lactarazulene (CLXXXVII) (reference *4*, p. 253), linderazulene (CLXXXVIII) (reference *4*, p. 261), artemazulene (CLXXXIX) (reference *4*, p. 275), and zierazulene (CXC) (reference *4*, p. 263) can be formed. To date, natural occurrence of azulenes has been established only for guaiazulene (CLXXXIV), guaiazulenic acid (CCLXX), lactaroviolin (CCLXXI), and an azulenaldehyde $C_{15}H_{12}O_3$ (*14*). Besides the sesquiterpene compounds mentioned, there are a number that contain large rings, such as humulene, caryophyllene, and the like. By means of rearrangements, formation of bridges, opening of rings, and oxidative loss of carbon atoms, irregularly constructed sesquiterpene compounds can arise (Table IX).

D. Diterpenes

Diterpene compounds of regular structure can occur in mono-, di-, and tricyclic forms. Besides this type (CCCXXXII), there is the irregular structure as exemplified by abietic acid (CCCXXXIII) and phyllocladene (CCCXXXIV).* From these basic skeletons other compounds can be derived by means of group migration or elimination and by ring opening, e.g., totarol (CCCLXVIII), nimbiol (CCCLXIX), podocarpic acid (CCCLXX), elliotinol (CCCLXXI), grayanol

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* See page 531.

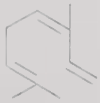
(CCCLXXVIII), etc. The well-known growth substance gibberellic acid (CCCLXXXIII) is formed by splitting off of a methyl group and ring contraction. The ring structure of atisine (CCCXCVI) can be thought of as resulting from ring expansion in the phyllocladene skeleton. Simultaneous ring contraction and expansion leads to the delpheline skeleton (CCCIC). Apart from the changes mentioned, others can, through dehydrogenation, lead to aromatic rings and to furans. The most important diterpene compounds known to date are shown in Table X.

E. TRITERPENES (*1, 4, 5, 9–11*)

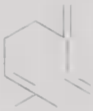
The triterpenes, formed from six isopentane units, can be distinguished from each other on the basis of differences in their skeleton, in the position of the ethylene linkages, or in the configuration of almost 20 substituent groups or subgroups (see Tables XI and XII). Squalene (CDX) is entirely aliphatic, ambrein (CDXI) tricyclic, and the onocerin group (CDXII) tetracyclic. A further large class of tetracyclic triterpenes has a skeleton similar to that of the steroids, and for this reason they are referred to as steroid triterpenes. They can be subdivided into the lanostane (CDI), euphol (CDII), and tirucallol (CDIII) groups. The pentacyclic triterpenes include particularly the skeletons of β-amyrin (CDVI), α-amyrin (CDVII), lupeol (CDVIII), and friedelin (CDIX). Recently representatives of the biogenetically interesting group of demethyl triterpenes (CDV) and a group with open ring A (CDIV) have been found. The aromatic compound gossypol (DXV) should also be included biogenetically among the triterpenes. For details of constitution, occurrence, and characteristics of the triterpenes, reference is made to Tables XI, XII, and XVI.

TABLE IV

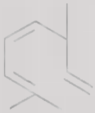
Terpene Compounds


β-Ocimene

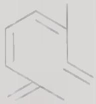
(XIX)


Myrcene

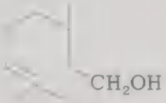
(XX)


Hymentherene

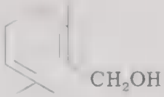
(XXI)


Cosmene

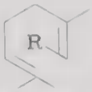
(XXII)


Citronellol

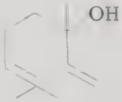
(XXIII)


Geraniol

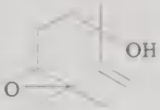
(XXIV)


R = HOCH$_2$
Nerol

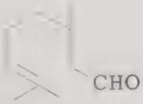
(XXV)


Linalool

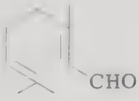
(XXVI)


Linalool
monoxide

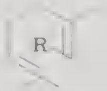
(XXVII)


Citronellal

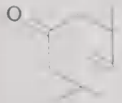
(XXVIII)


Geranial
(citral *a*)

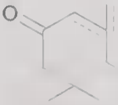
(XXIX)


R = OHC
Neral
(citral *b*)

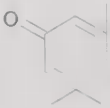
(XXX)


2, 6-Dimethyl
oct. 7-en-4-one

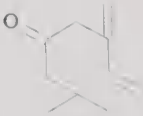
(XXXI)


Tagetone

(XXXII)

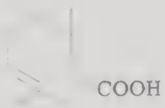

Ocimenone

(XXXIII)


Myrcenone

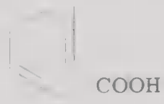
(XXXIV)

TABLE IV *(continued)*



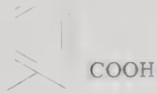
Citronellic
acid

(XXXV)



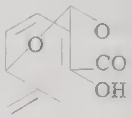
Geranic
acid

(XXXVI)



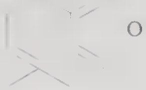
Dehydrogeranic
acid

(XXXVII)



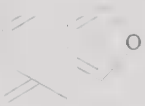
Mesogentiogenin

(XXXVIII)



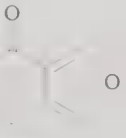
Perillene

(XXXIX)



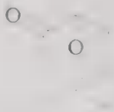
α-Clausenane

(XL)



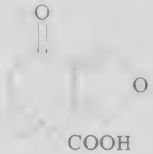
Perilla
ketone

(XLI)



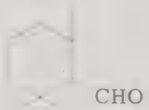
Elsholtzia
ketone

(XLII)



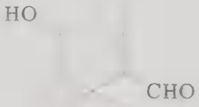
Batatic
acid

(XLIII)



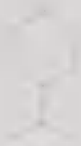
Safranal

(XLIV)



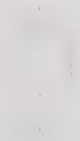
4-Hydroxy-*β*-
cyclocitral

(XLV)

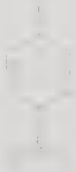


p-Menthane

(XLVI)

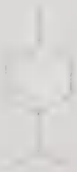


Limonene (XLVII)
dl-Dipentene (XLVIII)



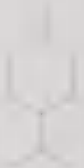
Isolimonene

(IL)



α-Terpinene

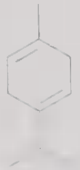
(L)



β-Terpinene

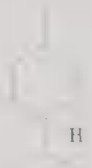
(LI)

TABLE IV *(continued)*



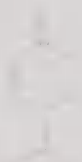
γ-Terpinene

(LII)



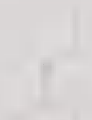
α-Phellandrene

(LIII)



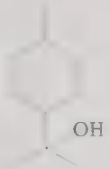
β-Phellandrene

(LIV)



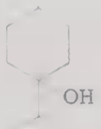
Terpinolene

(LV)



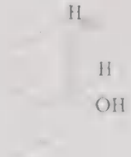
p-Menthanol-(8)

(LVI)



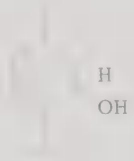
p-Menthanol-(4)

(LXI)



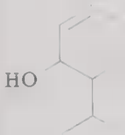
l-Menthol

(LVIII)



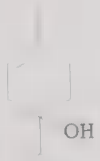
d-Neomenthol

(LIX)



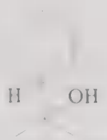
Piperitol

(LX)



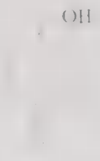
Terpineol-(4)

(LXI)



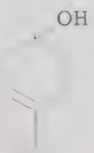
(+)-α-Terpineol

(LXII)



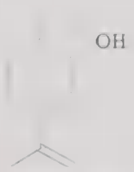
γ-Terpineol

(LXIII)



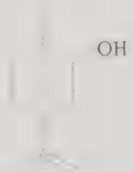
Terpinenol-(1)

(LXIV)



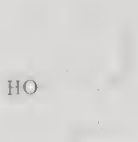
d-Dihydrocarveol

(LXV)



l-Neodihydrocarveol

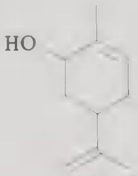
(LXVI)



Isopulegol

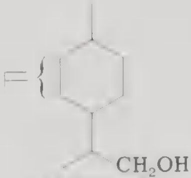
(LXVII)

TABLE IV *(continued)*



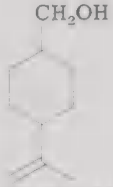
Carveol

(LXVIII)



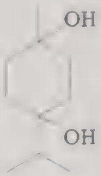
Artemisol

(LXIX)



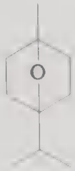
Perillyl alcohol

(LXX)



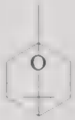
p-Menthane diol-(1,4)

(LXXI)



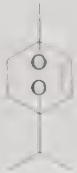
1,4-Cineole

(LXXII)



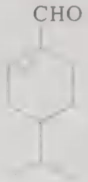
1,8-Cineole

(LXXIII)



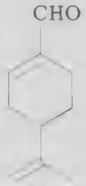
Ascaridole

(LXXIV)



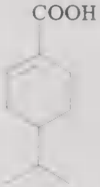
Phellandral

(LXXV)



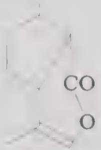
Perillaldehyde

(LXXVI)



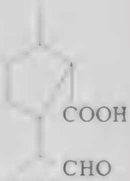
Phellandric acid

(LXXVII)



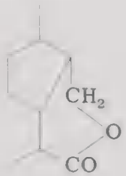
Nepetalactone

(LXXVIII)



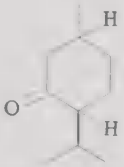
Nepetalic acid

(LXXIX)



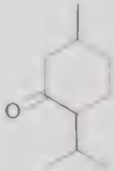
Iridomyrmecin

(LXXX)



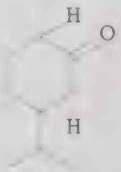
(+)-Menthone

(LXXXI)



Isomenthone

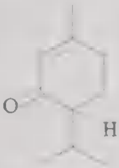
(LXXXII)



l-Carvomenthone

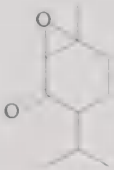
(LXXXIII)

TABLE IV *(continued)*



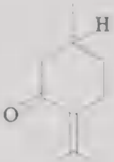
Piperitone

(LXXXIV)



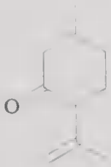
Piperitone oxide

(LXXXV)



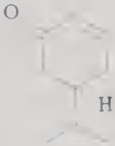
(+)-Pulegone

(LXXXVI)



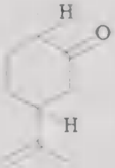
Isopulegone

(LXXXVII)



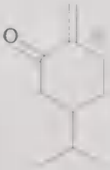
(-)-Carvotan acetone

(LXXXVIII)



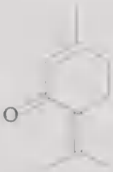
(-)-Dihydrocarvone

(LXXXIX)



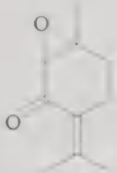
α-Santolinenone

(XC)



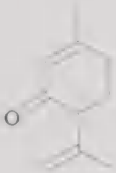
Piperitenone

(XCI)



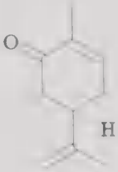
Piperitenone oxide

(XCII)



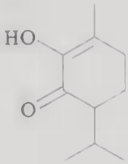
Isopiperitenone

(XCIII)



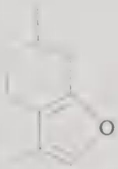
(-)-Carvone

(XCIV)



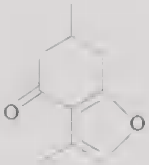
Diosphenol

(XCV)



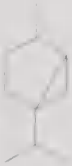
Menthofuran

(XCVI)



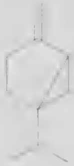
Evodone

(XCVII)



Salvene

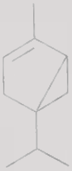
(XCVIII)



Sabinene

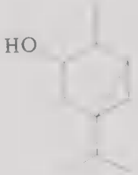
(IC)

TABLE IV *(continued)*



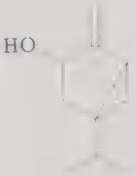
d-α-Thujene

(C)



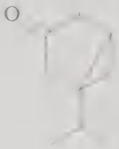
Thujyl alcohol

(CI)



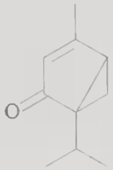
Sabinol

(CII)



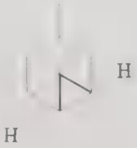
Thujone

(CIII)



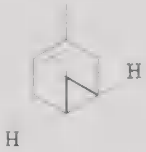
Umbellulone

(CIV)



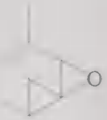
d-Δ^4-Carene

(CV)



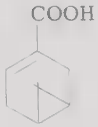
Δ^3-Carene

(CVI)



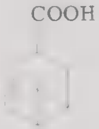
Δ^3-Carene-5, 6-epoxide

(CVII)



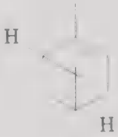
Chaminic acid

(CVIII)



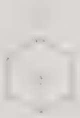
Chamic acid

(CIX)



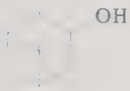
(+)-α -Pinene

(CX)



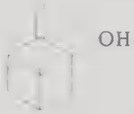
β-Pinene

(CXI)



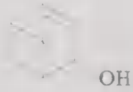
(-)-Pinocampheol

(CXII)



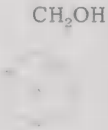
(-)-Pinocarveol

(CXIII)



Verbenol

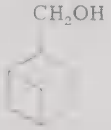
(CXIV)



Myrtenol

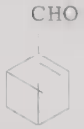
(CXV)

TABLE IV *(continued)*



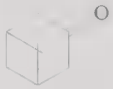
Benihiol

(CXVI)



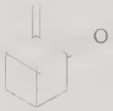
Myrtenal

(CXVII)



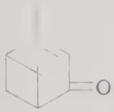
Pinocamphone

(CXVIII)



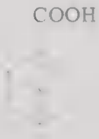
Pinocarvone

(CXIX)



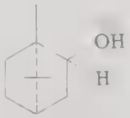
Verbenone

(CXX)



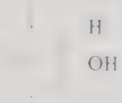
Myrtenic acid

(CXXI)



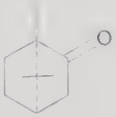
(-)-Borneol

(CXXII)



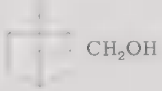
Isoborneol

(CXXIII)



(+)-Camphor

(CXXIV)

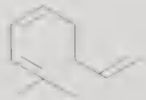


Teresantalol

(CXXV)

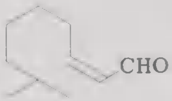
TABLE V

Irregularly Constructed and Terpenoid Aromatic Compounds



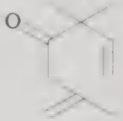
Cryptotaenene

(CXXVI)



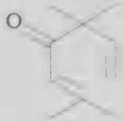
8-Methylnonen-2-al-(1)

(CXXVII)



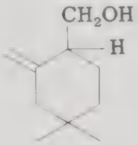
Artemisia ketone

(CXXVIII)



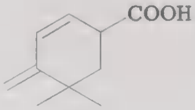
Isoartemisia ketone

(CXXIX)



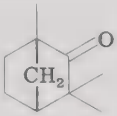
Cyclolavandulol

(CXXX)



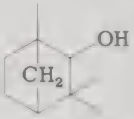
Shonanic acid

(CXXXI)



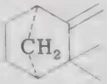
(+)-Fenchone

(CXXXII)



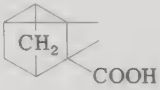
Fenchyl alcohol

(CXXXIII)



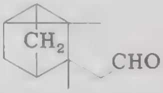
(+)-Camphene

(CXXXIV)



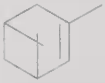
Teresantalic acid

(CXXXV)



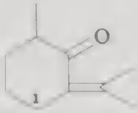
Nortricycloeka santalal

(CXXXVI)

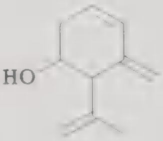


Orthodene

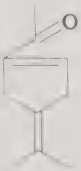
(CXXXVII)



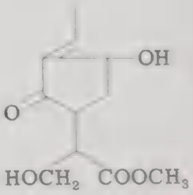
β-Santolinenone
(or CO in 1)
(CXXXVIII)



Carquejol
(CXXXIX)

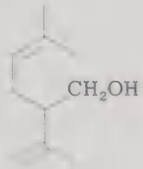


(3)-Isopropylidene-1-acetylcyclopentene-5
(CXL)

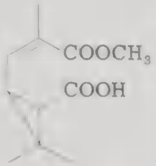


Loganetin
(CXLI)

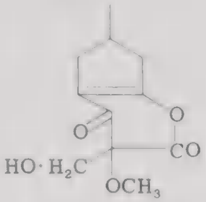
TABLE V (*continued*)



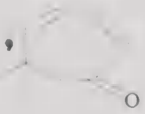
(-)-Lavandulol
(CXLII)



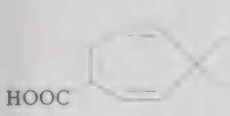
Chrysanthemum dicarboxylic acid methyl ester
(CXLIII)



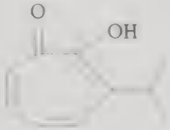
Verbenalol
(CXLIV)



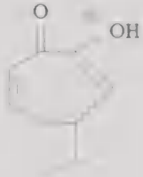
Eucarvone
(CXLV)



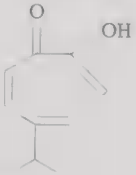
Thujic acid
(CXLVI)



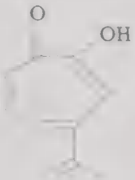
α-Thujaplicin
(CXLVII)



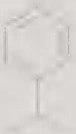
β-Thujaplicin
(CXLVIII)



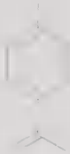
γ-Thujaplicin
(CIL)



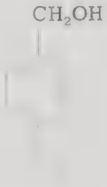
β-Dolabrin
(CL)



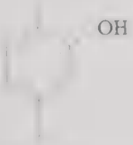
p-Cymene
(CLI)



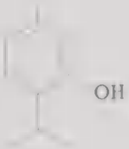
1-Methyl-4-isopropenylbenzene
(CLII)



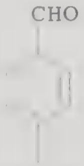
Cuminyl alcohol
(CLIII)



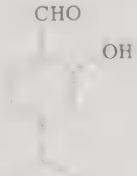
Carvacrol
(CLIV)



Thymol
(CLV)

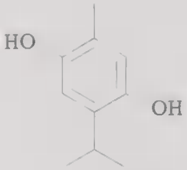


Cuminaldehyde
(CLVI)



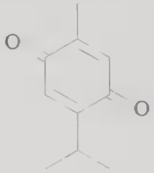
Macropone
(CLVII)

TABLE V *(continued)*



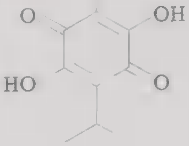
Thymohydro quinone

(CLVIII)



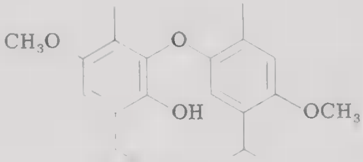
Thymoquinone

(CLIX)



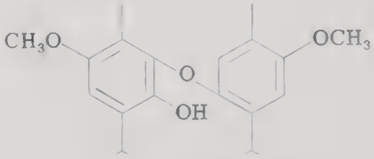
Dihydroxy thymoquinone

(CLX)



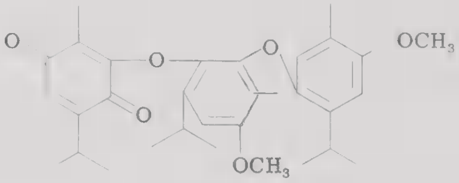
Heyderiol

(CLXI)



Libocedrol

(CLXII)

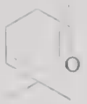


3-Libocedroxy-thymoquinone

(CLXIII)

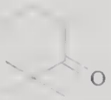
TABLE VI

Degraded Terpene Compounds



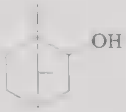
Methylheptenone

(CLXIV)



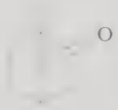
1,1,3-Trimethylcyclohexanone-(2)

(CLXV)



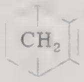
Santenol

(CLXVI)



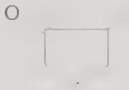
Santenone

(CLXVII)



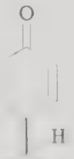
Santene

(CLXVIII)



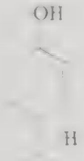
1,1,3-Trimethylcyclopentanone-(4)

(CLXIX)



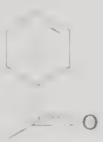
Cryptone

(CLXX)



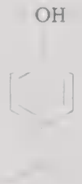
Cryptol

(CLXXI)



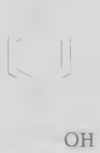
1-Methyl-4-acetylcyclohexene-(6)

(CLXXII)



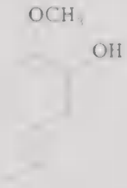
Australol

(CLXXIII)



α-*p*-Tolyl ethyl alcohol

(CLXXIV)

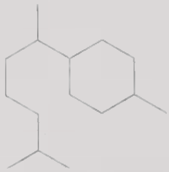


Chamenol

(CLXXV)

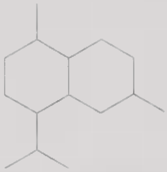
TABLE VII

Types of Sesquiterpenes and Dehydration Products



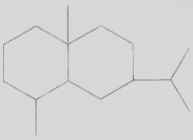
Bisabolene type

(CLXXVI)



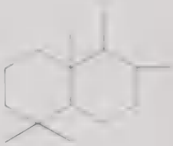
Cadinene type

(CLXXVII)



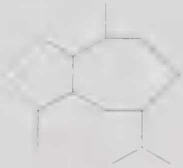
Selinene type

(CLXXVIII)



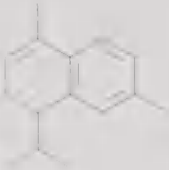
Iresin type

(CLXXIX)



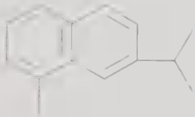
Guaiol type

(CLXXX)



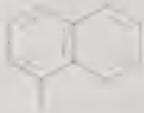
Cadalene

(CLXXXI)



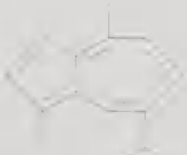
Eudalene

(CLXXXII)



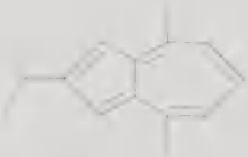
1, 5-Dimethyl naphthalene

(CLXXXIII)



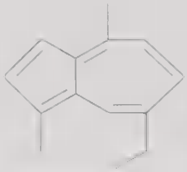
Guaiazulene

(CLXXXIV)



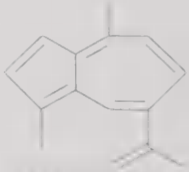
Vetivazulene

(CLXXXV)



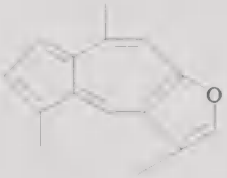
Chamazulene

(CLXXXVI)



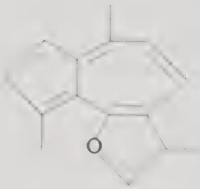
Lactarazulene

(CLXXXVII)



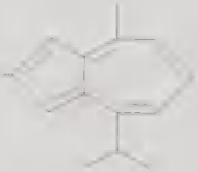
Linderazulene

(CLXXXVIII)



Artemazulene

(CLXXXIX)

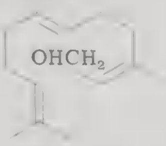


Zierazulene

(CXC)

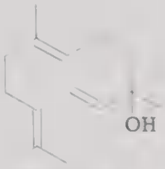
TABLE VIII

Sesquiterpene Compounds



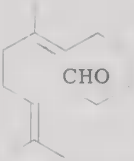
Farnesol

(CXCI)



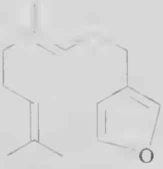
Nerolidol

(CXCII)



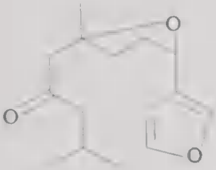
Farnesal

(CXCIII)

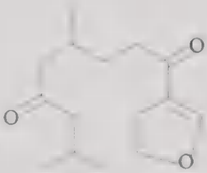


Dendrolasin

(CXCIV)

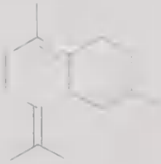


(CXCV) Ngaione (*trans*)
(CXCVI) Ipomeamarone (*cis*)



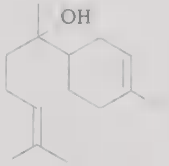
Myoporone

(CXCVII)



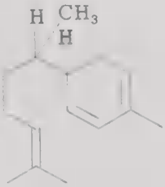
Bisabolene

(CXCVIII)



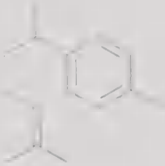
Bisabolol

(CIC)



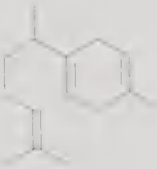
(-)-Zingiberene

(CC)



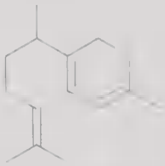
α-Curcumene

(CCI)



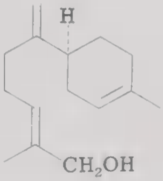
β-Curcumene

(CCII)



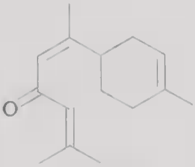
γ-Curcumene

(CCIII)



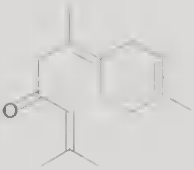
Lanceol

(CCIV)



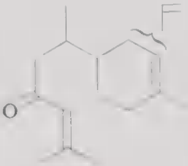
α-Atlantone

(CCV)



γ-Atlantone

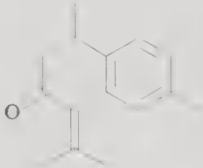
(CCVI)



Turmerone

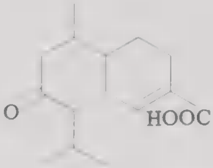
(CCVII)

TABLE VIII *(continued)*



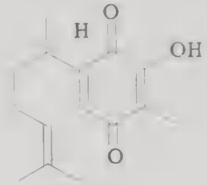
ar-Turmerone

(CCVIII)



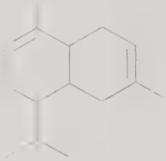
Todomatsu acid

(CCIX)



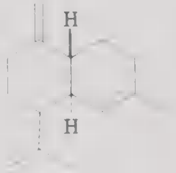
Perezone

(CCX)



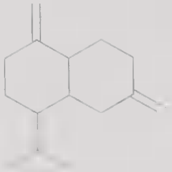
α-Cadinene

(CCXI)



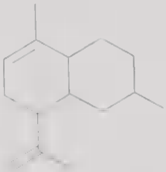
γ-Cadinene

(CCXII)



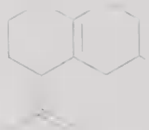
ε-Cadinene

(CCXIII)



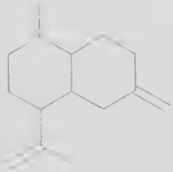
Sesquiterpene from vetiver oil

(CCXIV)



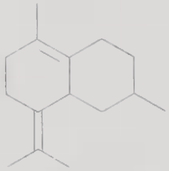
Damarin

(CCXV)



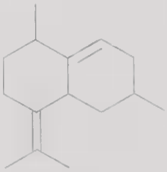
Sesquibenihene

(CCXVI)



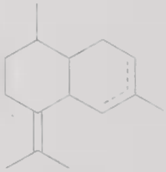
Metrosiderene

(CCXVII)



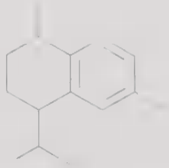
Eupatene

(CCXVIII)



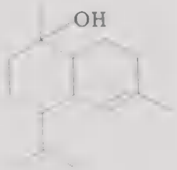
Kijanene

(CCXIX)



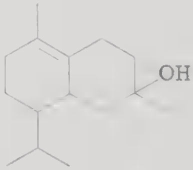
Calamenene

(CCXX)



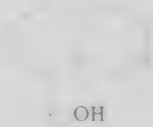
α-Cadinol

(CCXXI)



δ-Cadinol

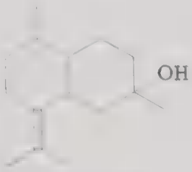
(CCXXII)



Kijanol

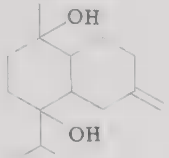
(CCXXIII)

TABLE VIII *(continued)*



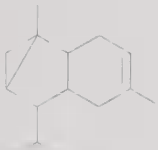
Torreyol

(CCXXIV)



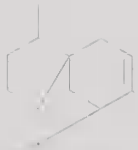
Calamenediol

(CCXXV)



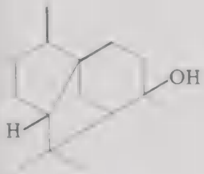
Copaene

(CCXXVI)



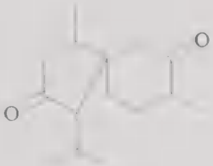
Cedrene

(CCXXVII)



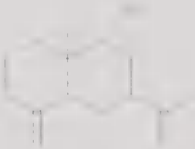
Cedrol

(CCXXVIII)



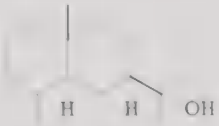
Acorone

(CCXXIX)



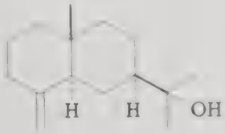
β-Selinene

(CCXXX)



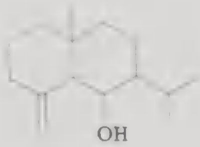
α-Eudesmol

(CCXXXI)



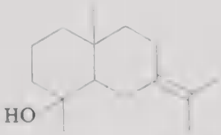
β-Eudesmol

(CCXXXII)



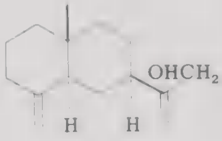
Junenol

(CCXXXIII)



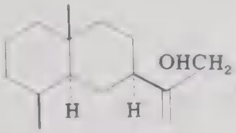
Juniper camphor

(CCXXXIV)



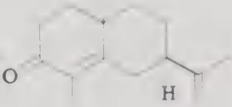
Sesquibenihiol (costol)

(CCXXXV)



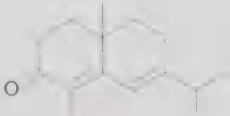
Agarol

(CCXXXVI)



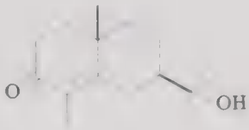
α-Cyperone

(CCXXXVII)



β-Cyperone

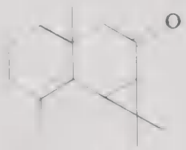
(CCXXXVIII)



Carissone

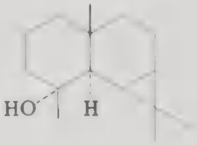
(CCXXXIX)

TABLE VIII *(continued)*



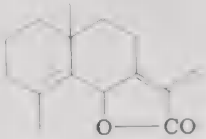
Germacrone
(compare CCLXIII)

(CCXL)



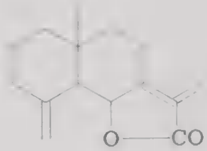
Maaliol

(CCXLI)



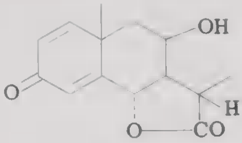
Atlantolactone

(CCXLII)



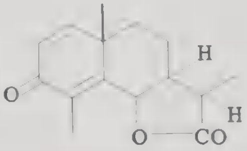
Isoatlantolactone

(CCXLIII)



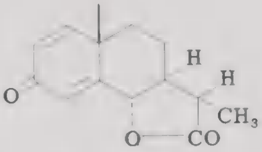
Artemesin

(CCXLIV)



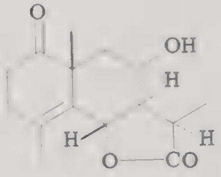
α-Santonin

(CCXLV)



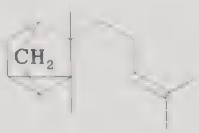
β-Santonin

(CCXLVI)



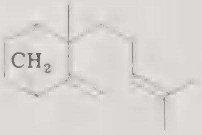
Ψ-Santonin

(CCXLVII)



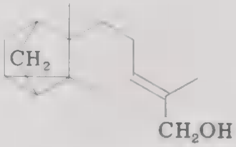
α-Santalene

(CCXLVIII)



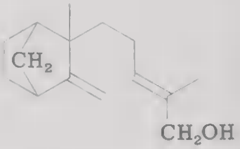
β-Santalene

(CCIL)



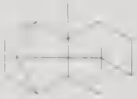
α-Santalol

(CCL)



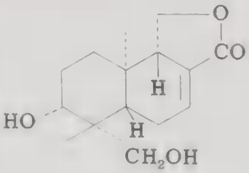
β-Santalol

(CCLI)



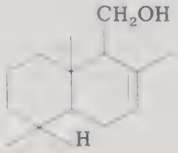
Longifolene

(CCLII)



Iresin

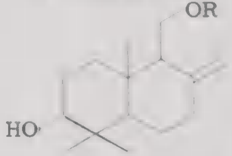
(CCLIII)



Drimenol

(CCLIV)

R = Cumaryl

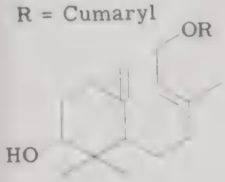


Farnesiferol A

(CCLV)

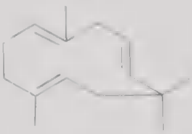
TABLE VIII *(continued)*

R = Cumaryl



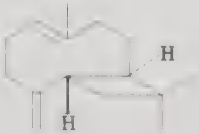
Farnesiferol B

(CCLVI)



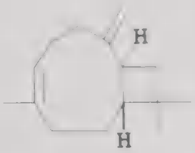
Humulene

(CCLVII)



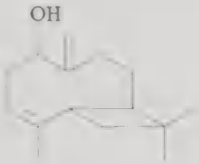
Caryophyllene (*trans*)

(CCLVIII)



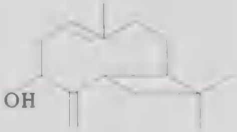
Isocaryophyllene (*cis*)

(CCLIX)



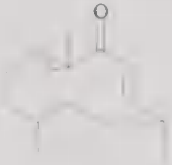
α-Betulenol

(CCLX)



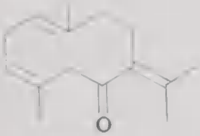
β-Betulenol

(CCLXI)



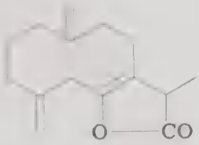
Zerumbone

(CCLXII)



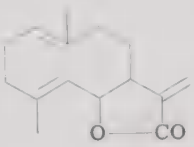
Germacrone
(compare CCXL)

(CCLXIII)



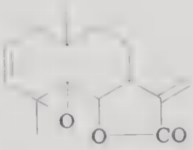
Aristolactone

(CCLXIV)



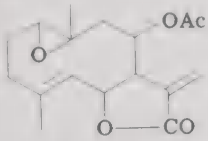
Costunolide

(CCLXV)



Parthenolide

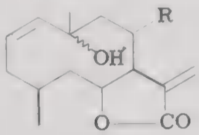
(CCLXVI)



Pyrethrosin

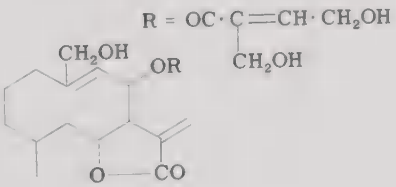
(CCLXVII)

R= -OOC · CH · (CH_3) · CH_2OH



Arctiopicrin

(CCLXVIII)

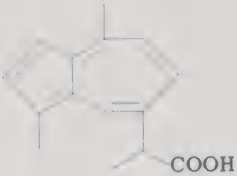


$R = OC \cdot \underset{\displaystyle CH_2OH}{C} = CH \cdot CH_2OH$

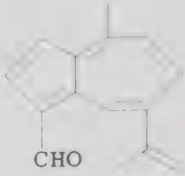
Cnicin

(CCLXIX)

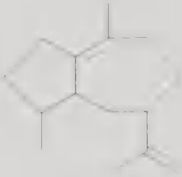
TABLE VIII *(continued)*



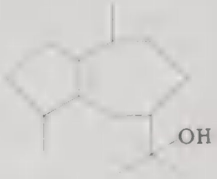
Guaiazulenic acid
(CCLXX)



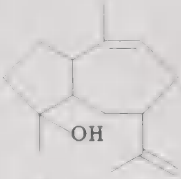
Lactaroviolin
(CCLXXI)



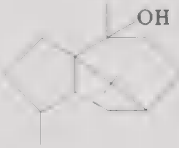
α-Chigadmarene
(CCLXXII)



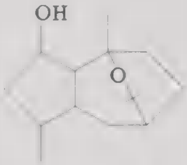
Guaiol
(CCLXXIII)



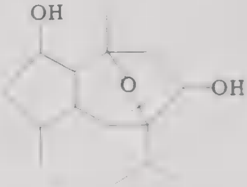
Partheniol
(CCLXXIV)



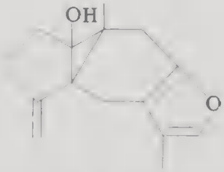
Patchouli alcohol
(CCLXXV)



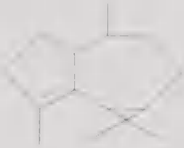
Kessyl alcohol
(CCLXXVI)



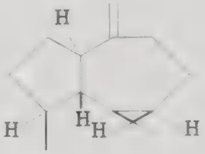
Kessoglycol
(CCLXXVII)



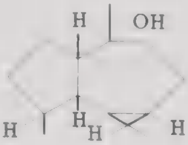
Linderene
(CCLXXVIII)



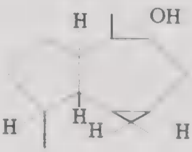
Gurjunene
(CCLXXIX)



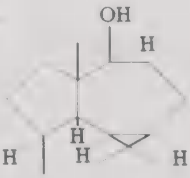
Aromadendrene
(CCLXXX)



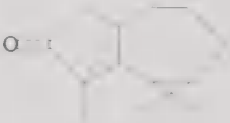
Ledol
(CCLXXXI)



Globulol
(CCLXXXII)

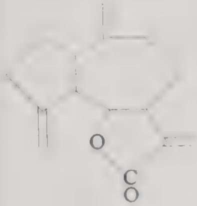


Viridiflorol
(CCLXXXIII)

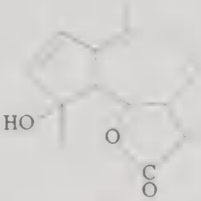


Cyclocolorenone
(CCLXXXIV)

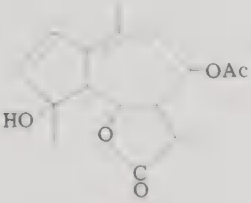
TABLE VIII *(continued)*



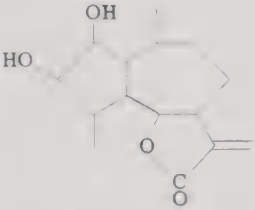
Dehydrocostus lactone
(CCLXXXV)



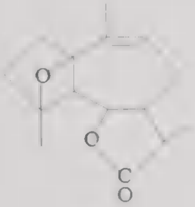
Artabasin
(CCLXXXVI)



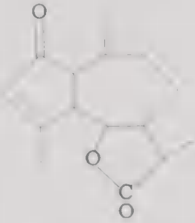
Matricin
(CCLXXXVII)



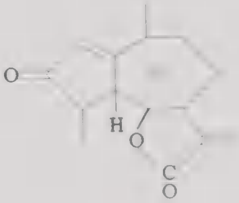
Geigerenin
(CCLXXXVIII)



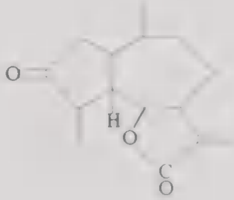
Arborescin
(CCLXXXIX)



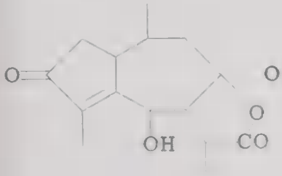
Carpesia lactone
(CCXC)



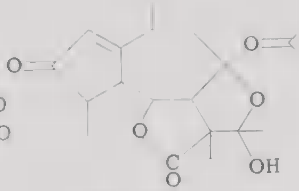
Ambrosin
(CCXCI)



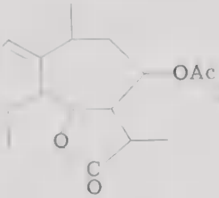
Damsin
(CCXCII)



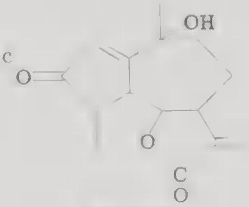
Geigerin
(CCXCIII)



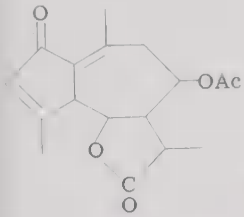
Tenulin
(CCXCIV)



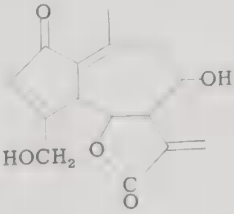
Isotenulin
(CCXCV)



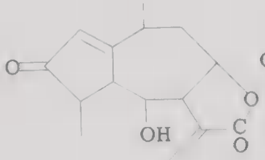
Parthenin
(CCXCVI)



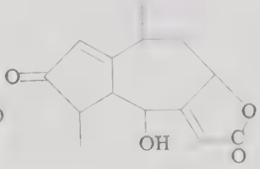
Matricarin
(CCXCVII)



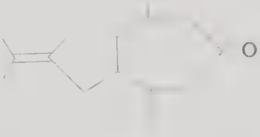
Lactucin
(CCXCVIII)



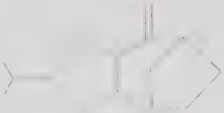
Helenalin (CCIC)
Balduilin (=Acetate)
(CCC)



Isohelenalin
(CCCI)



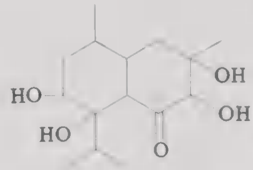
β-Vetivone
(CCCII)



Tricyclovetivene
(CCCIII)

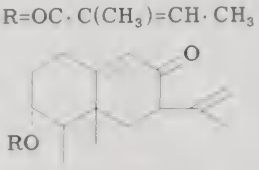
TABLE IX

Sesquiterpenes: Irregularly Constructed and Degradation Compounds



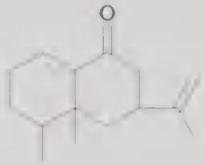
Laserol

(CCCIV)



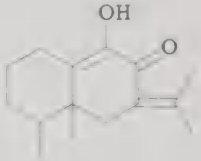
Petasin

(CCCV)



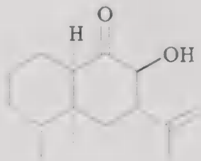
Eremophilone

(CCCVI)



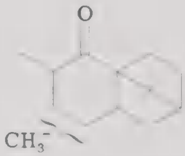
Hydroxyeremophilone

(CCCVII)



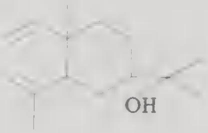
Hydroxydihydro-eremophilone

(CCCVIII)



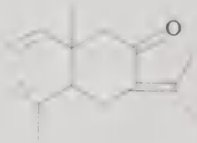
Aristolone

(CCCIX)



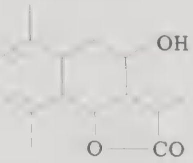
Elemol

(CCCX)



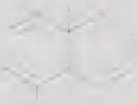
α-Elemenone

(CCCXI)



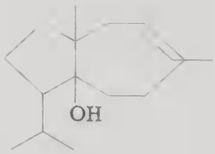
Temisin

(CCCXII)



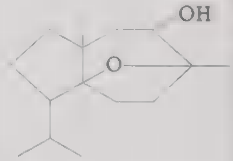
Geijerene

(CCCXIII)



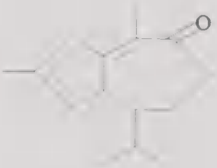
Carotol

(CCCXIV)



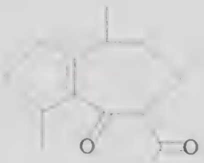
Daucol

(CCCXV)



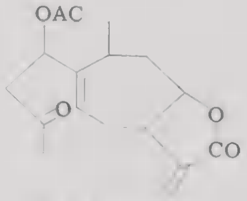
Zierone

(CCCXVI)



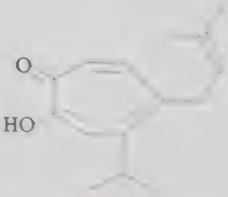
Dammar diketone

(CCCXVII)



Xanthinin

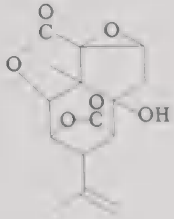
(CCCXVIII)



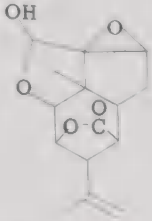
Nootkatin

(CCCXIX)

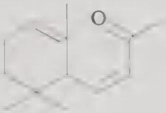
TABLE IX *(continued)*



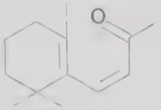
Picrotoxinin
(CCCXX)



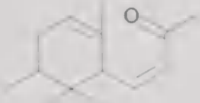
Coriamyrtione
(CCCXXI)



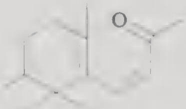
α-Ionone
(CCCXXII)



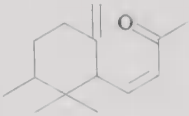
β-Ionone
(CCCXXIII)



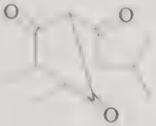
α-Irone
(CCCXXIV)



β-Irone
(CCCXXV)



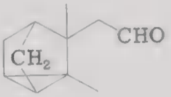
γ-Irone
(CCCXXVI)



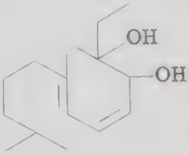
Calythrone
(CCCXXVII)

R = OC · CH = CH · CH_3

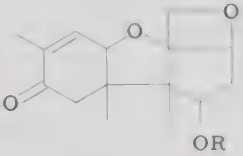
R = β- D -Glucosyl



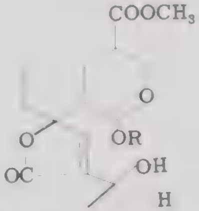
Nortricycloekasantalal
(CCCXXVIII)



Grifolin
(CCCXXIX)

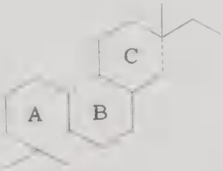


Trichothecin
(CCCXXX)

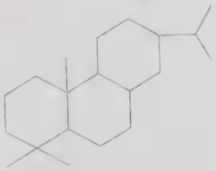


Plumieride
(CCCXXXI)

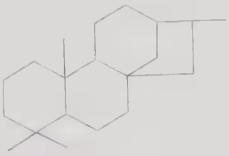
Basic diterpene skeletons



(CCCXXXII)



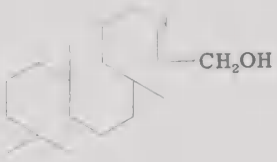
(CCCXXXIII)



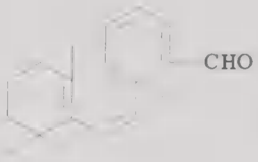
(CCCXXXIV)

TABLE X

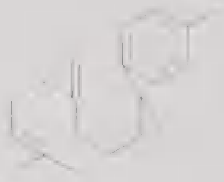
Diterpene Compounds



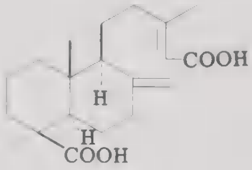
Phytol
(CCCXXXV)



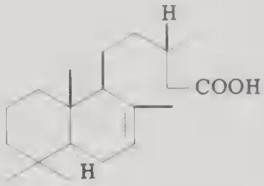
Vitamin A
(CCCXXXVI)



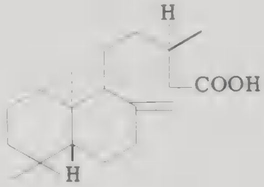
Artemisene
(CCCXXXVII)



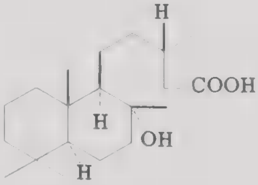
Agathene
dicarboxylic acid
(CCCXXXVIII)



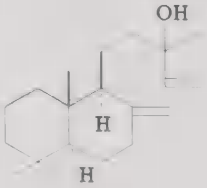
Cativic acid
(CCCXXXIX)



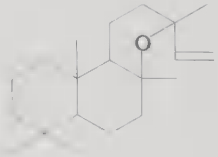
Eperuic acid
(CCCXL)



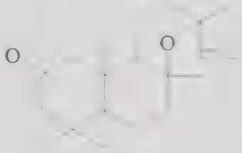
Labdanolic acid
(CCCXLI)



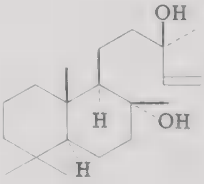
Manool
(CCCXLII)



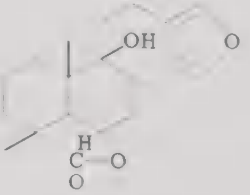
Manoyl oxide
(CCCXLIII)



Ketomanoyl oxide
(CCCXLIV)

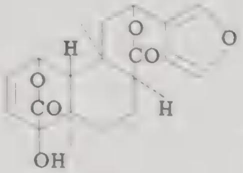


Sclareol
(CCCXLV)



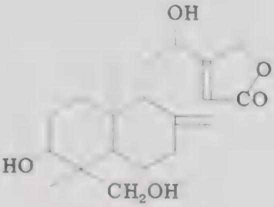
Marrubiin
(CCCXLVI)

TABLE X *(continued)*



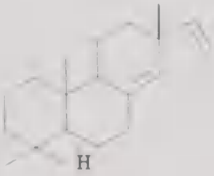
Columbin

(CCCXLVII)



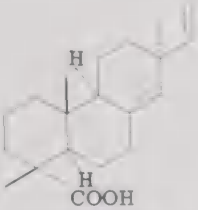
Andrographolide

(CCCXLVIII)



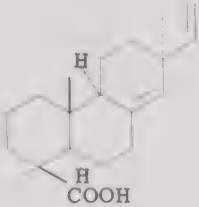
Rimuene

(CCCIL)



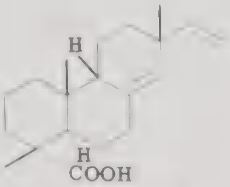
Dextropimaric acid

(CCCL)



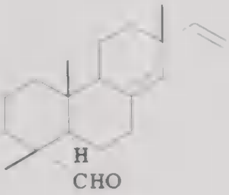
Sandaracopimaric acid

(CCCLI)



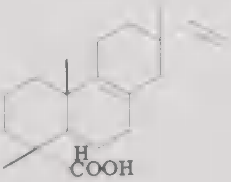
Isodextropimaric acid

(CCCLII)



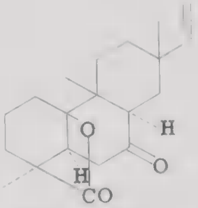
Isodextropimarinal

(CCCLIII)



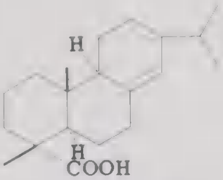
7-Isodextropimaric acid

(CCCLIV)



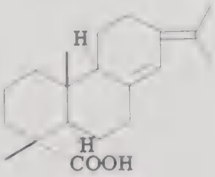
Rosenonolactone

(CCCLV)



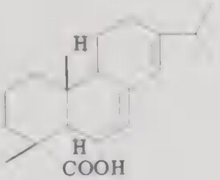
Levopimaric acid

(CCCLVI)



Neoabietic acid

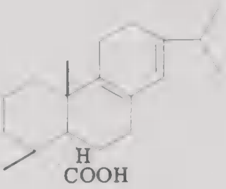
(CCCLVII)

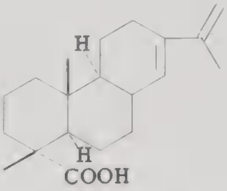


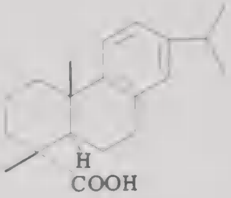
Abietic acid

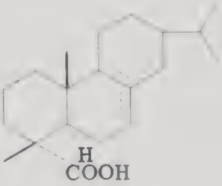
(CCCLVIII)

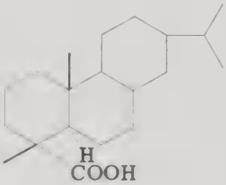
TABLE X *(continued)*

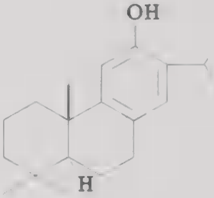

Palustric acid
(CCCLIX)

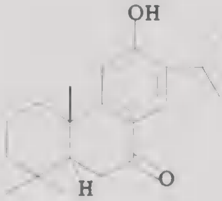

Teloabietic acid
(CCCLX)

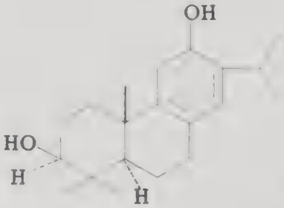

Dehydroabietic acid
(CCCLXI)

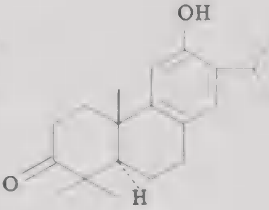

Dihydroabietic acid
(CCCLXII)

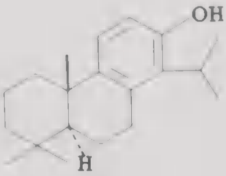

Tetrahydroabietic acid
(CCCLXIII)

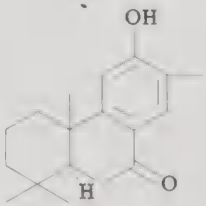

Ferruginol
(CCCLXIV)


9-Ketoferruginol
(CCCLXV)


Hinokiol
(CCCLXVI)


Hinokione
(CCCLXVII)


Totarol
(CCCLXVIII)


Nimbiol
(CCCLXIX)

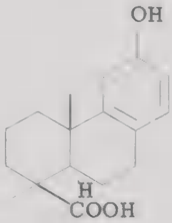
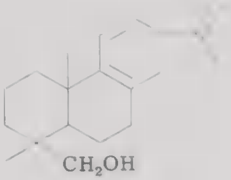
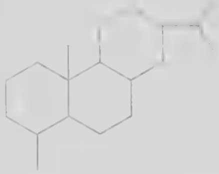

Podocarpic acid
(CCCLXX)

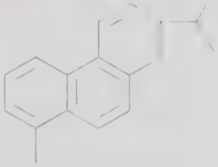
TABLE X *(continued)*



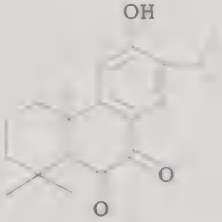
Elliotinol
(CCCLXXI)



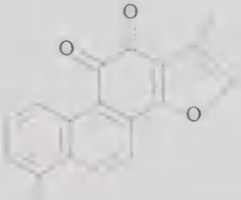
Fichtelite
(CCCLXXII)



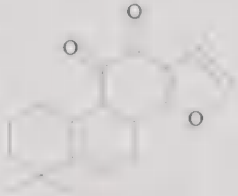
Retene
(CCCLXXIII)



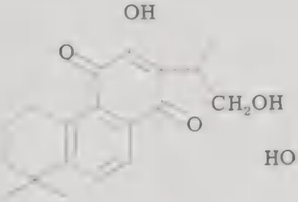
Xanthoperol
(CCCLXXIV)



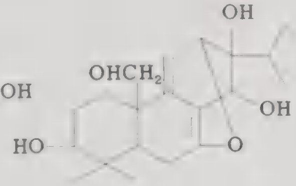
Tanshinone I
(CCCLXXV)



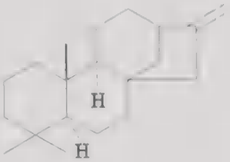
Tanshinone II
(CCCLXXVI)



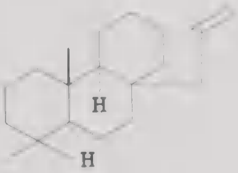
Cryptotanshinone
(CCCLXXVII)



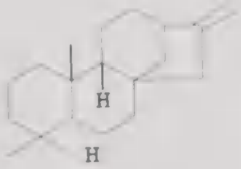
Grayanol
(CCCLXXVIII)



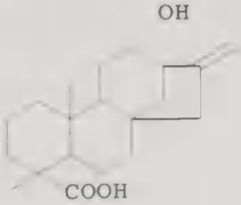
Phyllocladene
(CCCLXXIX)



Kaurene
(CCCLXXX)

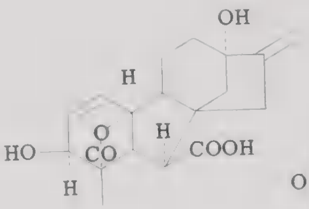


Mirene
(CCCLXXXI)

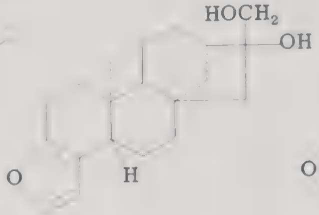


Steviol
(CCCLXXXII)

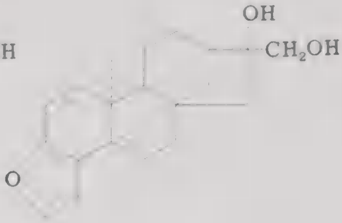
TABLE X *(continued)*



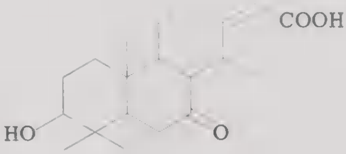
Gibberellic acid
(CCCLXXXIII)



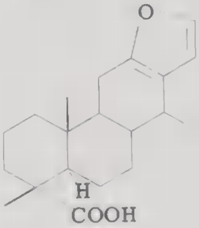
Cafestol
(CCCLXXXIV)



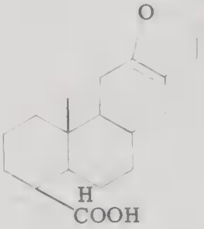
Kahweol
(CCCLXXXV)



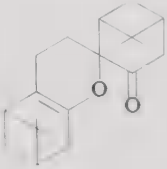
Cassaic acid
(CCCLXXXVI)



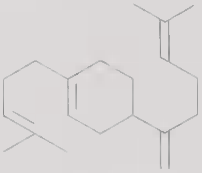
Vinhaticoic acid
(CCCLXXXVII)



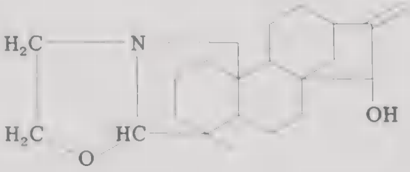
Vuocapenic acid
(CCCLXXXVIII)



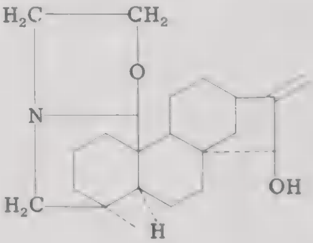
Aritasone
(CCCLXXXIX)



α-Camphorene
(CCCXC)

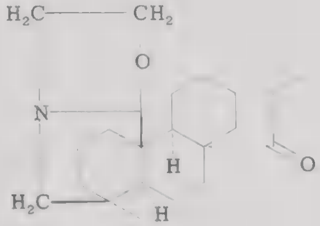


Garryine
(CCCXCI)



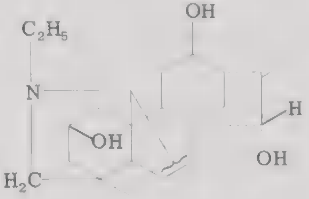
Veatchine
(CCCXCII)

TABLE X (*continued*)



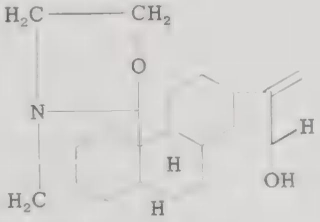
Cuauchichicine

(CCCXCIII)



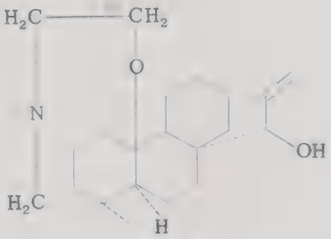
Napelline

(CCCXCIV)



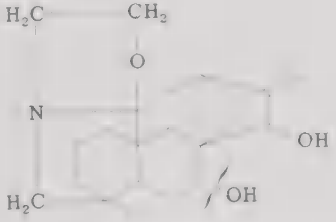
Garryfoline

(CCCXCV)



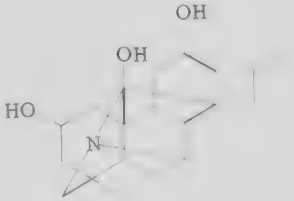
Atisine

(CCCXCVI)



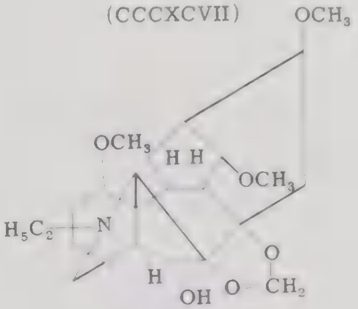
Ajaconine

(CCCXCVII)



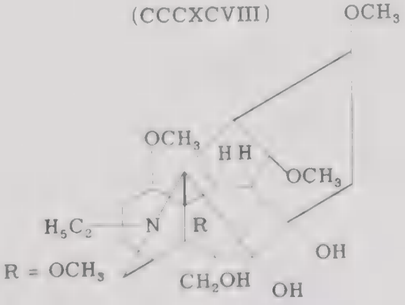
Hetisine

(CCCXCVIII)



Delpheline

(CCCIC)

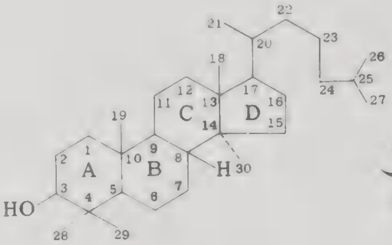


Lycoctonine

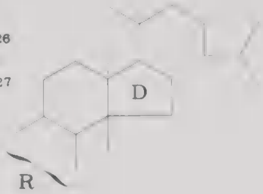
(CD)

TABLE XI

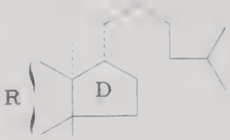
The Most Important Groups of Triterpenes



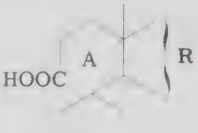
Lanostane group
(CDI)



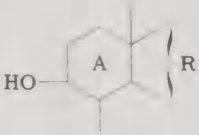
Euphol group
(CDII)



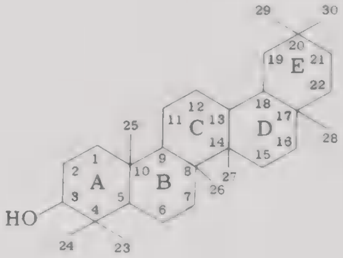
Tirucallol group
(CDIII)



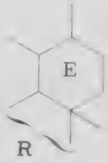
Nyctanthic
acid group
(CDIV)



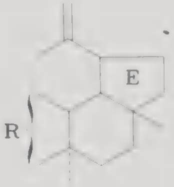
Demethyl
triterpene group
(CDV)



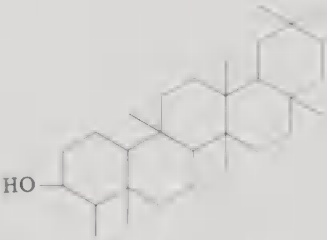
β-Amyrin group
(CDVI)



α-Amyrin group
(CDVII)



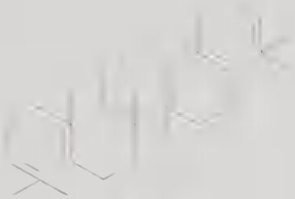
Lupeol group
(CDVIII)



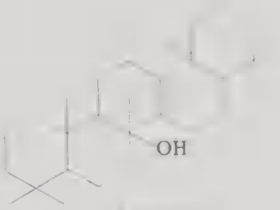
Friedelin group
(CDIX)

TABLE XII

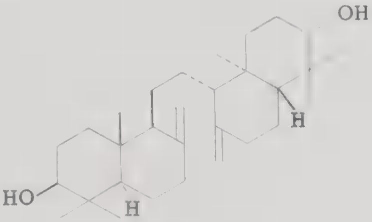
Triterpenes



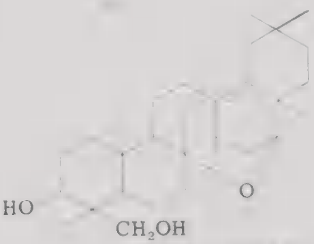
Squalene
(CDX)



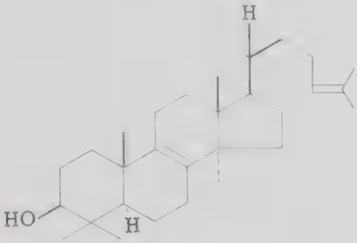
Ambrein
(CDXI)



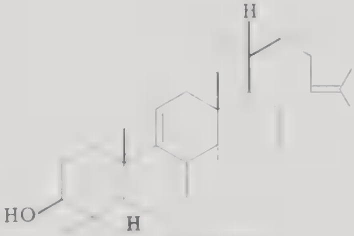
α-Onocerin
(CDXII)



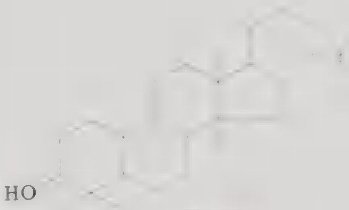
Soysapogenol II
(CDXIII)



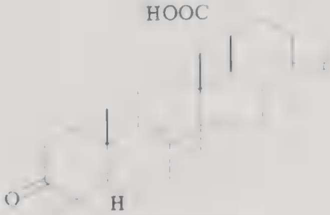
Lanosterol
(CDXIV)



Agnosterol
(CDXV)

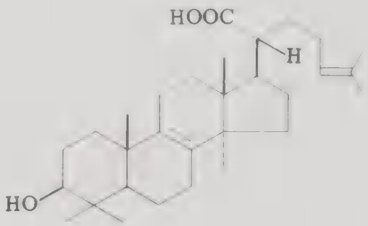


Parkeol
(CDXVI)

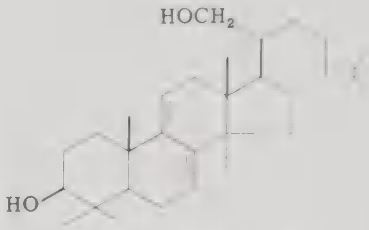


Pinicolic acid
(CDXVII)

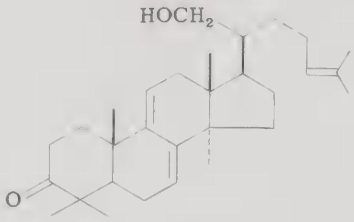
TABLE XII *(continued)*



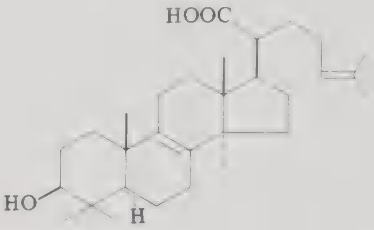
Trametenolic acid B
(CDXVIII)



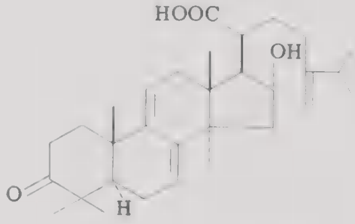
Lanosta 7,9(11),24-triene-3β, 21-diol
(CDXIX)



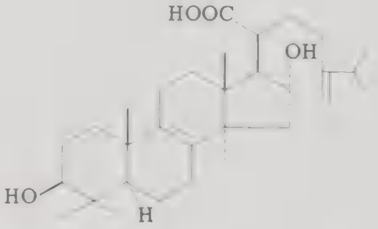
21-Hydroxylanosta-7,9 (11),24-trien-3-one
(CDXX)



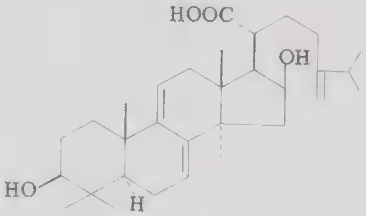
3β-Hydroxylanosta-8,24-dien-21-oic acid
(CDXXI)



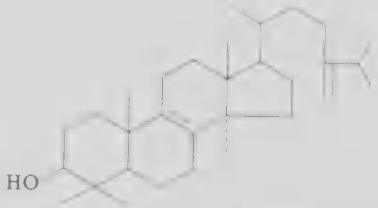
Polyporenic acid C
(CDXXII)



Tumulosic acid
(CDXXIII)

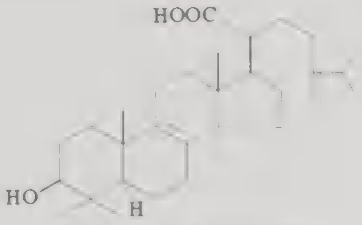


Dehydrotumulosic acid
(CDXXIV)

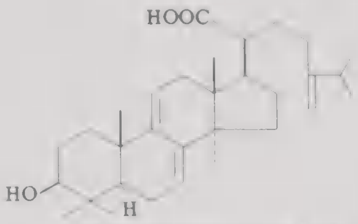


Obtusifoldienol
(CDXXV)

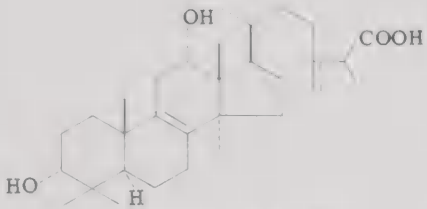
TABLE XII *(continued)*



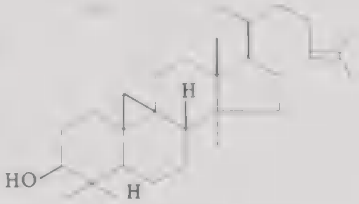
Eburicoic acid
(CDXXVI)



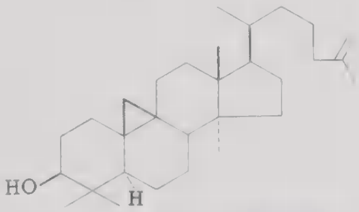
Dehydroeburicoic acid
(CDXXVII)



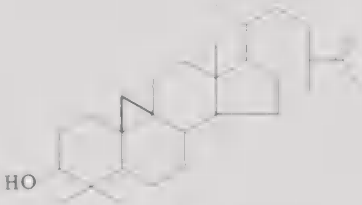
Polyporenic acid A
(CDXXVIII)



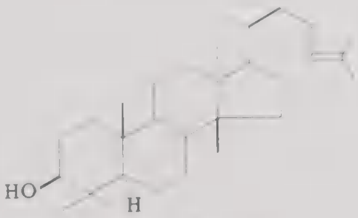
Cycloartenol
(CDXXIX)



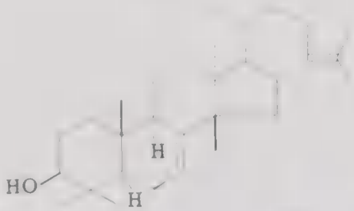
β-Cycloorystenol
(CDXXX)



Cyclolaudenol
(CDXXXI)

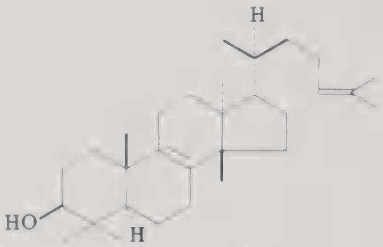


Euphol
(CDXXXII)

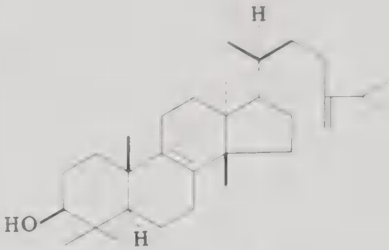


Butyrospermol
(CDXXXIII)

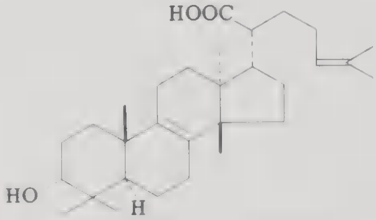
TABLE XII *(continued)*



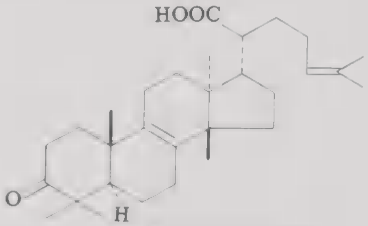
Tirucallol
(CDXXXIV)



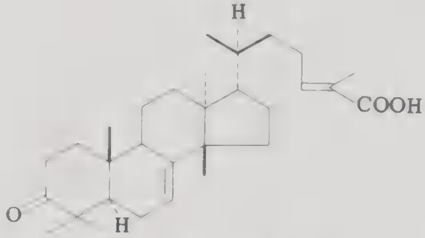
Euphorbol
(CDXXXV)



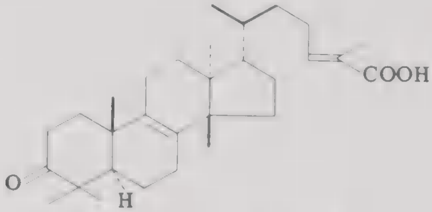
α-Elemolic acid
(CDXXXVI)



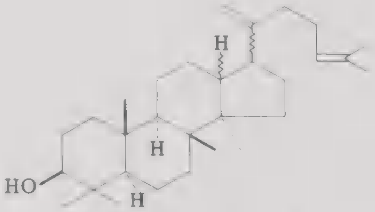
β-Elemonic acid
(CDXXXVII)



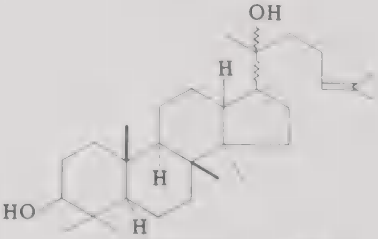
Masticadienonic acid
(CDXXXVIII)



Isomasticadienonic acid
(CDXXXIX)

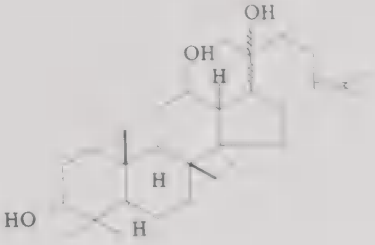


Dammadienol
(CDXL)

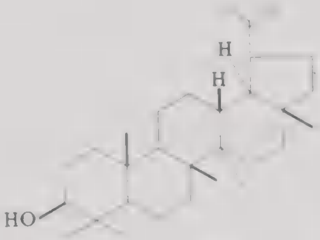


Dammarenediol
(CDXLI)

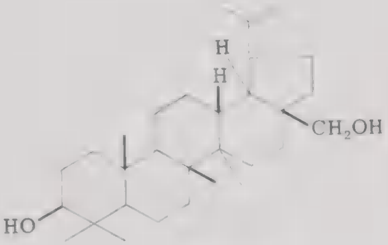
TABLE XII *(continued)*



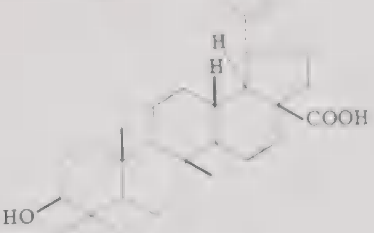
Betulafolientriol
(CDXLII)



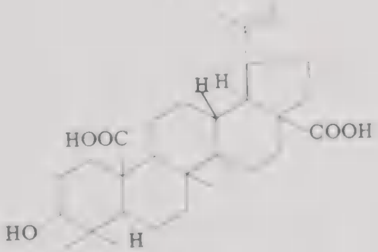
Lupeol
(CDXLIII)



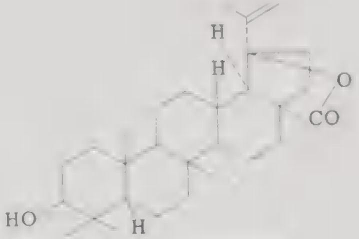
Betulin
(CDXLIV)



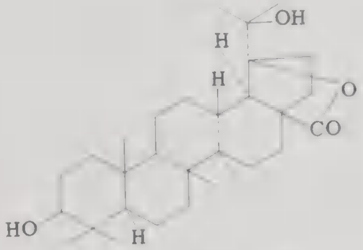
Betulinic acid
(CDXLV)



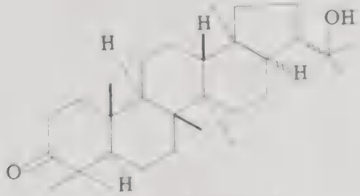
Melaleucinic acid
(CDXLVI)



Thurberogenin
(CDXLVII)

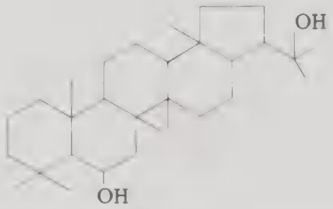


Stellatogenin
(CDXLVIII)

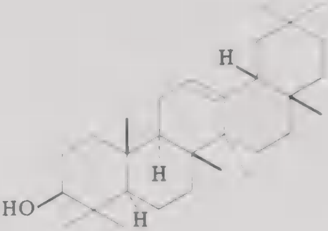


Hydroxyhopanone
(CDIL)

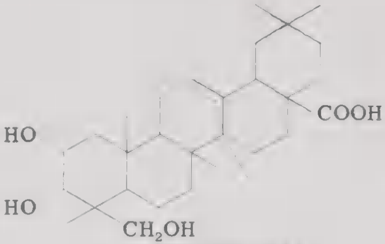
TABLE XII *(continued)*



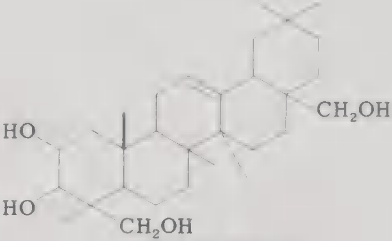
Zeorin
(CDL)



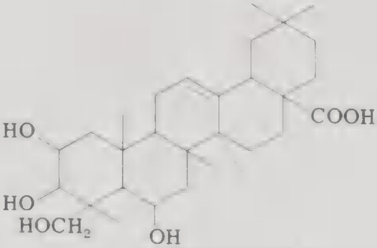
β-Amyrin
(CDLI)



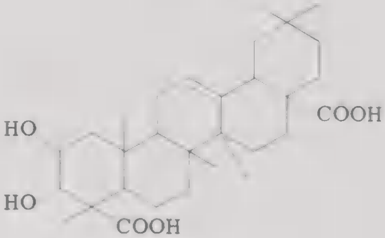
Arjunolic acid
(CDLII)



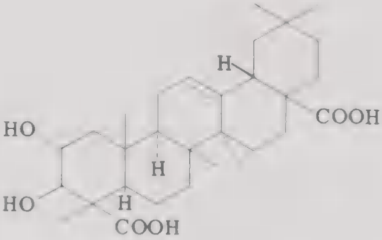
Barringtogenol
(CDLIII)



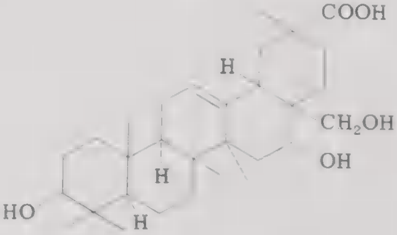
Terminolic acid
(CDLIV)



Barringtogenolic acid
(CDLV)

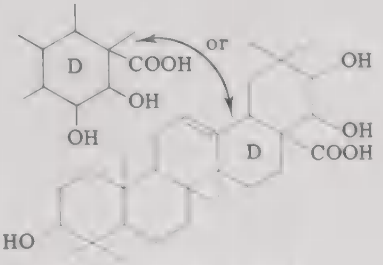


Medicagenic acid
(CDLVI)

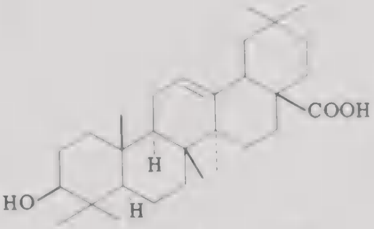


Myrtillogenic acid
(CDLVII)

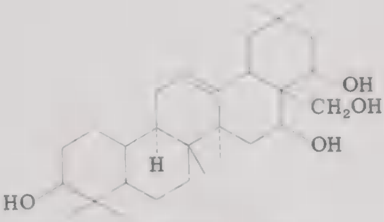
TABLE XII *(continued)*



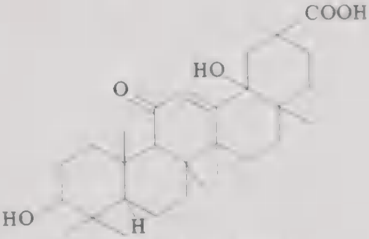
Entagenic acid
(CDLVIII)



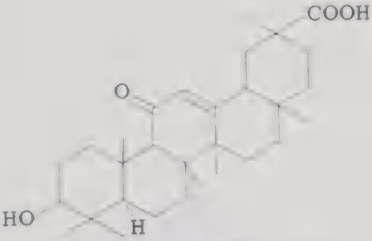
Oleanolic acid
(CDLIX)



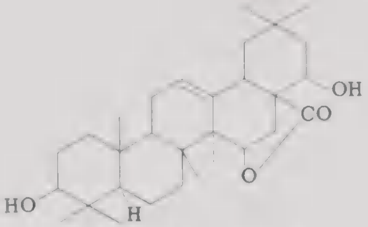
Chichipegenin
(CDLX)



Glabrinic acid
(CDLXI)

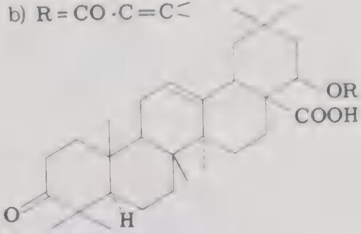


Glycyrrhetinic acid
(CDLXII)



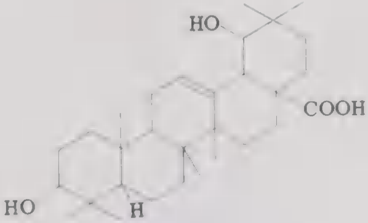
Dumortierigenin
(CDLXIII)

a) $R = CO\cdot C(CH_3){=}CH\cdot CH_3$
b) $R = CO\cdot C{=}C<$



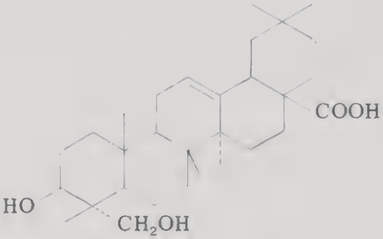
a) Lantadene A
= Rehmannic acid
(CDLXIV)

b) Lantadene B
(CDLXV)

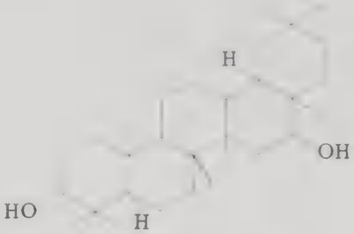


Siaresinolic acid
(CDLXVI)

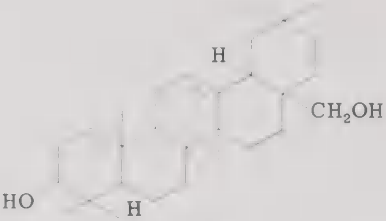
TABLE XII *(continued)*



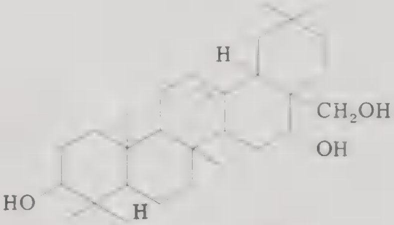
Hederagenin
(CDLXVII)



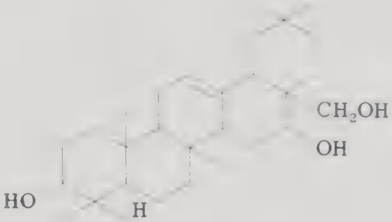
Maniladiol
(CDLXVIII)



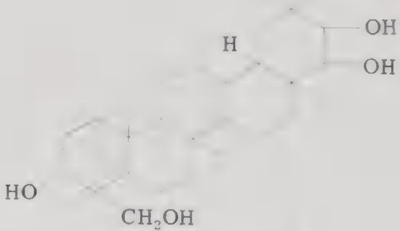
Erythrodiol
(CDLXIX)



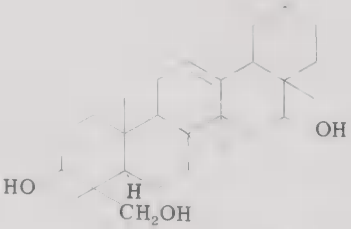
Primula genin A
(CDLXX)



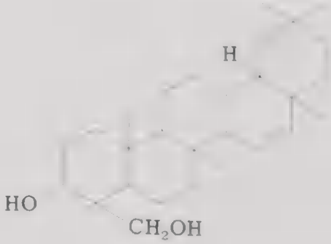
Longispinogenin
(CDLXXI)



Soysapogenol A
(CDLXXII)

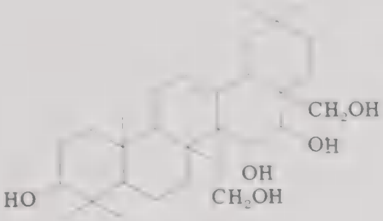


Soysapogenol B
(CDLXXIII)

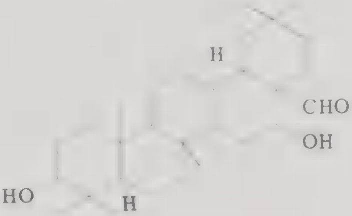


Soysapogenol C
(CDLXXIV)

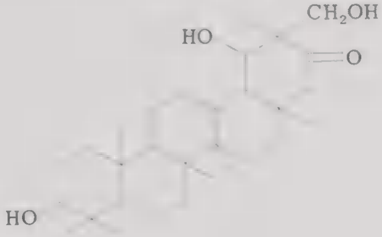
TABLE XII *(continued)*



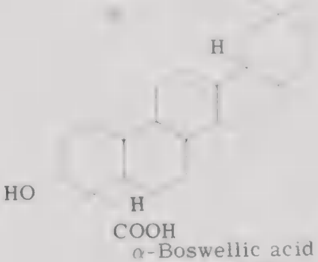
A$_1$-Barrigenol
(CDLXXV)



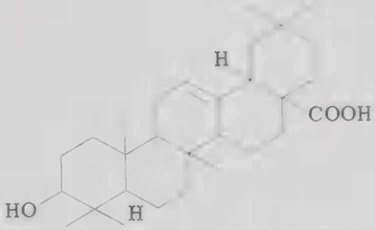
Gummosogenin
(CDLXXVI)



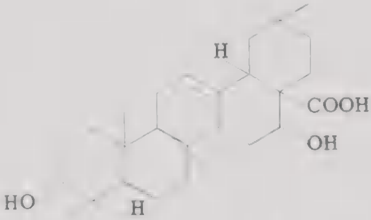
Gratiogenin
(CDLXXVII)



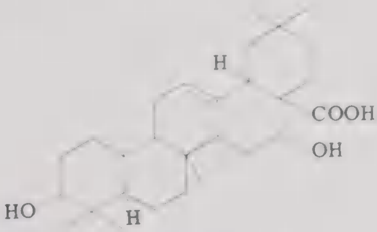
α-Boswellic acid
(CDLXXVIII)



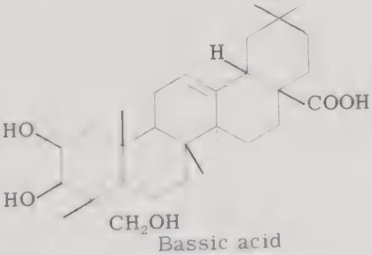
Sumaresinolic acid
(CDLXXIX)



Echinocystic acid
(CDLXXX)

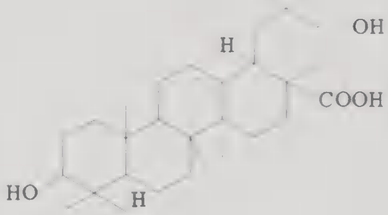


Cochalic acid
(CDLXXXI)

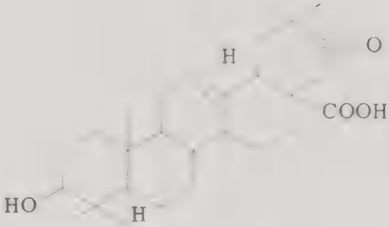


Bassic acid
(CDLXXXII)

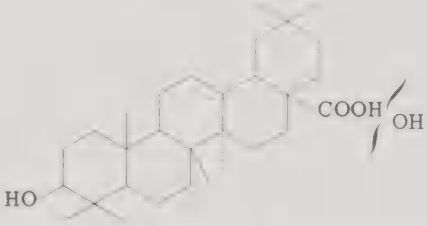
TABLE XII *(continued)*



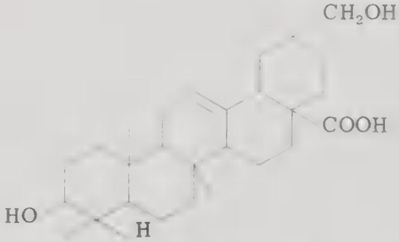
Machaerinic acid
(CDLXXXIII)



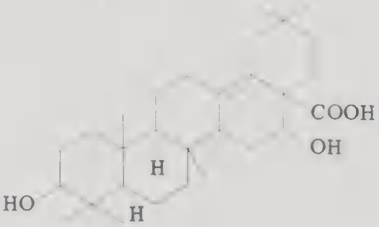
Machaeric acid
(CDLXXXIV)



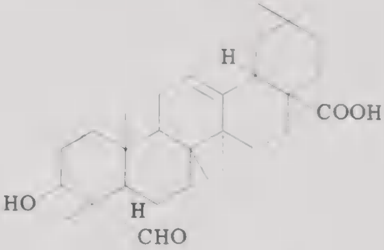
Crataegolic acid
(CDLXXXV)



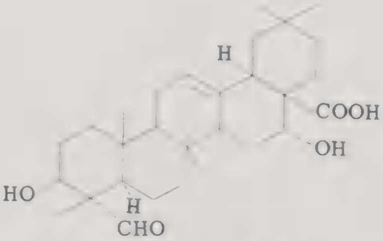
Queretaroic acid
(CDLXXXVI)



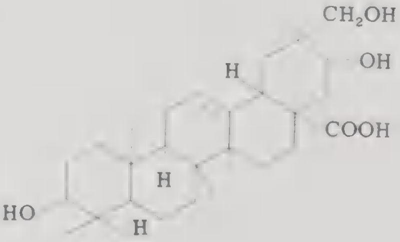
Albigenic acid
(CDLXXXVII)



Gypsogenin
(CDLXXXVIII)



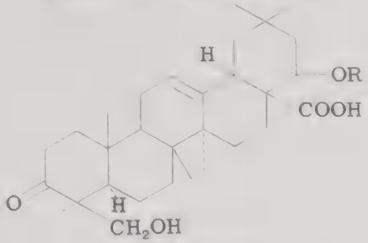
Quillaic acid
(CDLXXXIX)



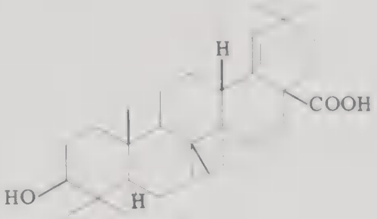
Treleasegenic acid
(XD)

TABLE XII *(continued)*

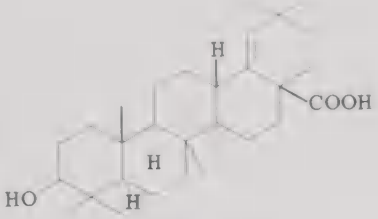
R = CO—C(CH$_3$)=CH·CH$_3$



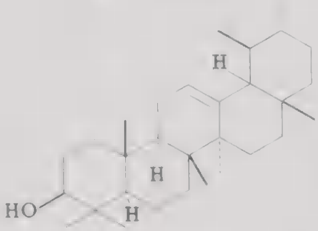
Icterogenin
(XDI)



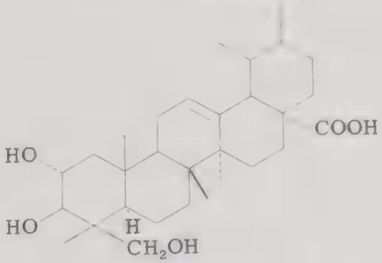
Morolic acid
(XDII)



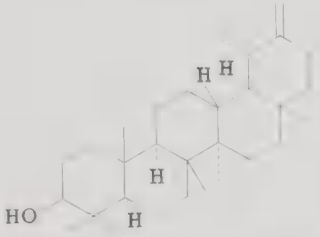
Germanicol
(XDIII)



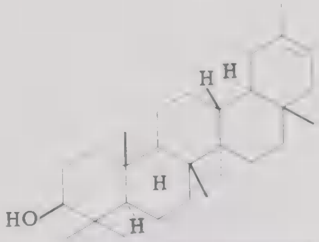
α-Amyrin
(XDIV)



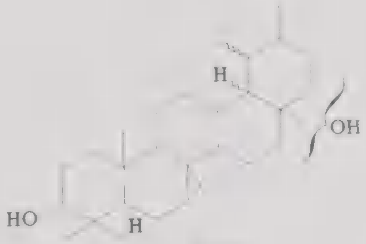
Asiatic acid
(XDV)



Taraxasterol
(XDVI)

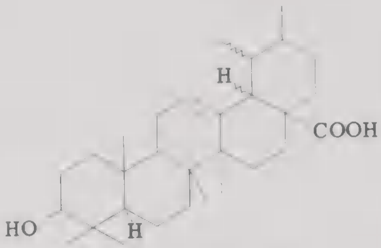


Ψ-Taraxasterol
(XDVII)

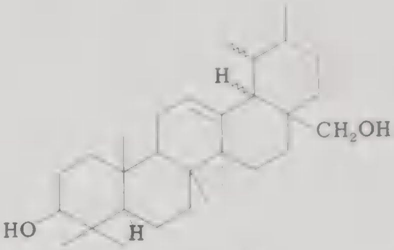


Brein
(XDVIII)

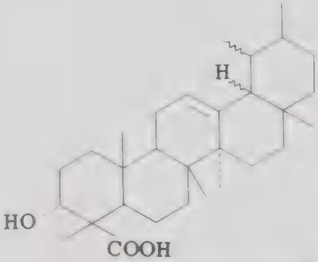
TABLE XII *(continued)*



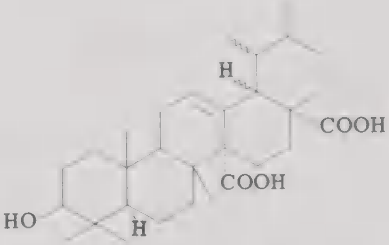
Ursolic acid
(ID)



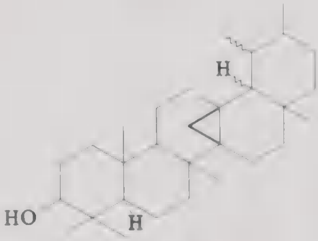
Uvaol
(D)



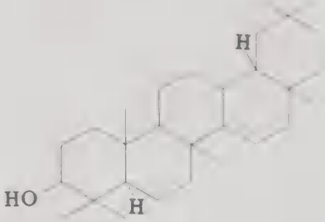
β-Boswellic acid
(DI)



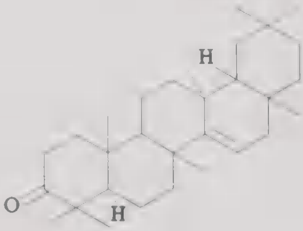
Quinovic acid
(DII)



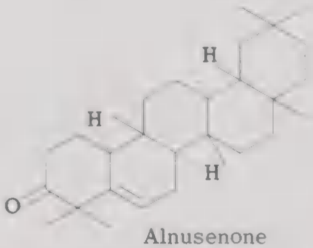
Phyllantol
(DIII)



Taraxerol
(DIV)

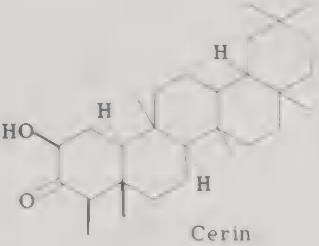


Taraxerone
(DV)

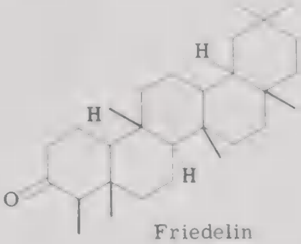


Alnusenone
(= Glutinone)
(DVI)

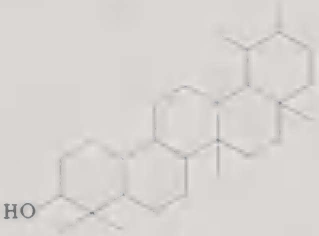
TABLE XII *(continued)*



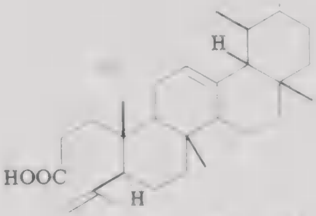
Cerin
(DVII)



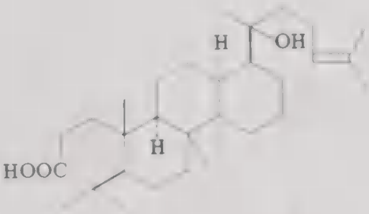
Friedelin
(DVIII)



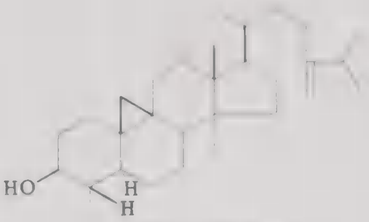
Bauerenol
(DIX)



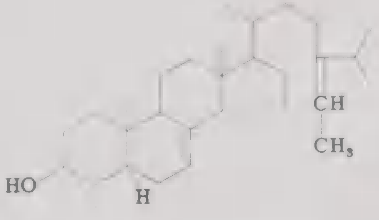
Nyctanthic acid
(DX)



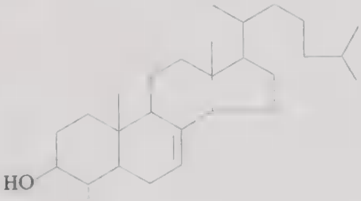
Dammarenolic acid
(DXI)



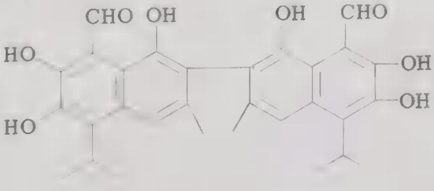
Cycloeucalenol
(DXII)



Citrostadienol
(DXIII)



Lophenol
(DXIV)



Gossypol
(DXV)

TABLE XIII

Monoterpenes and Related Compounds: Occurrence, Formulas, and Physical Constants

Compound	Number	Formula	Source	Physical constants	References
Artemisia ketone	(CXXVIII)	$C_{10}H_{16}O$	*Artemisia annua* L.	$[\alpha]_D$ −2.04°; b.p. 182–183°	[(*5*), p. 48; (*1*), Vol. I, p. 105]
Artemisol	(LXIX)	$C_{10}H_{18}O$	*Artemisia tridentata*	b.p.$_{17}$ 115°	[(*5*), p. 127]
Ascaridole	(LXXIV)	$C_{10}H_{16}O_2$	*Chenopodium ambrosioides* var. *anthelminticum* Gray; *C. multifidum; C. hircinum*	b.p.$_9$ 97°	[(*1*), Vol. I, p. 446 (*5*)p. 223]
Australol	(CLXXIII)	$C_9H_{12}O$	*Eucalyptus* spp.	m.p. 61°	[(*5*), p. 74]
Batatic acid	(XLIII)	$C_{10}H_{12}O_4$	*Ceratostomella fimbriata* (on potatoes)	$[\alpha]_D^{10}$ +17.5°; m.p. 88.5–89.5°	[(*5*), p. 705]
Benihiol	(CXVI)	$C_{10}H_{18}O$	*Chamaecyparis formosensis*	$[\alpha]_D^{22}$ +23.4°; b.p. 234–237°	[(*5*), p. 132]
Borneol	(CXXII)	$C_{10}H_{18}O$	In 150 species (Pinaceae, Labiatae, Compositae); (−): *Abies sibirica* Lebed.	(−): $[\alpha]_D^{20}$ −36°; m.p. 203–204°	[(*1*), Vol. II, p. 349]
Camphene	(CXXXIV)	$C_{10}H_{16}$	(+): *Abies sibirica* Lebed. (−): *Pinus palustris* Miller (In 85 species (Pinaceae, Gramineae, Rutaceae, Labiatae, Compositae, etc.)	(+): m.p. 45–46° (−): m.p. 50°	[(*1*), Vol. II, p. 280]
Camphor	(CXXIV)	$C_{10}H_{16}O$	(+): *Cinnamomum camphora* sieb.; *Ocimum canum* var. *camphoratum* (−): *Salvia triloba* L.; *Tanacetum vulgare; Achillea moschata* L. (Pinaceae, Zingiberaceae, Lauraceae, Labiatae, Compositae)	$[\alpha]_D$ (±) 40.24°; m.p. 178–178.8°	[(*1*), Vol. II, p. 373; (*5*), p. 278]
Δ^4-Carene (+)	(CV)	$C_{10}H_{16}$	*Pinus sylvestris* L.; *Dacrydium* spp.; *Eucalyptus* spp.	$[\alpha]_D$ +82.6°; b.p. 160–166°	[(*1*), Vol. II, p. 73]
Δ^3-Carene	(CVI)	$C_{10}H_{16}$	(+): *Pinus longifolia* Roxb.; *P. sylvestris* L.; *Andropogon iwarancusa* Jones. (Pinaceae, Gramineae)	(+): $[\alpha]_D$ +14.6°; b.p. 167–170°	[(*1*), Vol. II, p. 64]

Δ^3-Carene-5,6-epoxide	(CVII)	$C_{10}H_{14}O$	*Zieria smithi* Andr.; *Pinus sylvestris* L.; *Piper cubeba* L.; *Eucalyptus rarifolia* Bailey	$[\alpha]_{5461}$ −88°; b.p.$_{14}$ 83–85°	[(*5*), p. 33]
Carquejol	(CXXXIX)	$C_{10}H_{14}O$	*Baccharis genistelloides* Pers.	$[\alpha]_D$ = 32.7° (CH_3OH); m.p. 37–38°	(*15*)
Carvacrol	(CLIV)	$C_{10}H_{14}O$	*Corydothymus capitatus* Reichb. f.	b.p. 237–238°	[(*1*), Vol. I, p. 147; (*5*), p. 77]
Carveol	(LXVIII)	$C_{10}H_{16}O$	*Carum carvi* L.	$[\alpha]_D^{25}$ +23.9°; m.p. 24–25°	[(*1*), Vol. I, p. 297]
l-Carvomenthone	(LXXXIII)	$C_{10}H_{18}O$	*Blumea malcolmii* Hook; *B. eriantha*	$[\alpha]_D$ −24.9°; b.p. 219–221°	[(*1*), Vol. I, p. 327]
Carvone	(XCIV)	$C_{10}H_{14}O$	(+): *Carum carvi* L.; *Anethum graveolens* L. (−): *Mentha spicata* Huds.; *M. crispa* L.; *dl:* Gingergrass oil	$[\alpha]_D^{20}$ (±)62°; b.p. 230–231°	[(*1*), Vol. I, p. 394]
Carvotan acetone (+)	(LXXXVIII)	$C_{10}H_{16}O$	Thuja oil; *Blumea malcolmii; B. eriantha; Pulicaria mauritanica*	$[\alpha]_D^{30}$ +59.5°; b.p. 228–229°	[(*1*), Vol. I, p. 338]
Chamenol	(CLXXV)	$C_{10}H_{14}O_2$	*Chamaecyparis taiwanensis*	m.p. 41–42°	[(*5*), p. 82]
Chamic acid	(CIX)	$C_{10}H_{14}O_2$	*Chamaecyparis nootkaensis*	$[\alpha]_D^{20}$ +257.5°; b.p.$_8$ 142°	[(*5*), p. 397]
Chaminic acid	(CVIII)	$C_{10}H_{14}O_2$	*Chamaecyparis nootkaensis*	$[\alpha]_D$ +6°; m.p. 104–105°	[(*5*), p. 398]
Chrysanthemum dicarboxylic acid methyl ester	(CXLIII)	$C_{11}H_{16}O_4$	Pyrethrin II	$[\alpha]_D^{18}$ +103.9° (CCl_4)	[(*5*), p. 387]
1,4-Cineole	(LXXII)	$C_{10}H_{18}O$	*Piper cubeba* L.; *Boldea fragrans* Juss; *Ormenis multicaulis*	b.p. 173–174°	[(*1*), Vol. I, p. 433]
1,8-Cineole	(LXXIII)	$C_{10}H_{18}O$	*Eucalyptus polybractea* R. T. Baker; *E. globulus; Melaleuca cajaputi* Roxb.; citronella oil	b.p. 176–177°	[(*1*), Vol. I, p. 423]

TABLE XIII (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
(+)-Citronellal	(XXVIII)	$C_{10}H_{18}O$	*Cymbopogon winterianus* Jowitt (Pinaceae, Gramineae, Rutaceae, Myrtaceae, Labiatae)	$[\alpha]_D$ +12.5°; b.p. 207–208°	[(*1*), Vol. I, p. 71]
Citronellol	(XXIII)	$C_{10}H_{20}O$	*Cymbopogon winterianus* Jowitt (Gramineae, Geraniaceae, Rutaceae, Myrtaceae, etc.)	(+): $[\alpha]_D^{20}$ +4.17°; b.p.224–224.5°	[(*1*), Vol. I, p. 26]
Citronellic acid	(XXXV)	$C_{10}H_{18}O_2$	(+): *Pelargonium graveolens* Ait.; *Callitris* spp. (−): *C. glauca; Thujopsis dolabrata; Chamaecyparis obtusa* Sieb. et Zucc.	(+): $[\alpha]_D$ +21°; b.p. 257°	[(*5*), p. 301]
α-Clausenane	(XL)	$C_{10}H_{12}O$	*Clausena wildenovii* Wght.	b.p. 177–178°	[(*5*), p. 701]
Cosmene	(XXII)	$C_{10}H_{14}$	*Amellus strigosus; Cosmos bipinnatus* (20–40% of oil)	b.p.$_{0.3}$ 30°; m.p. −1°	[(*5*), p. 23]
Cryptol	(CLXXI)	$C_9H_{16}O$	(+)*Phellandrium aquaticum* L.	b.p.$_2$ 72°	[(*2*), Vol. II, p. 299]
Cryptone	(CLXX)	$C_9H_{14}O$	(+): *Phellandrium aquaticum* L. (−): *Pinus pumilio; Eucalyptus* spp.	(+): $[\alpha]_D$+64.6°	[(*1*), Vol. I, p. 334]
Cryptotaenene	(CXXVI)	$C_{10}H_{16}$	*Cryptotaenia japonica* Harsk.	b.p.$_{15}$ 67°	[(*5*), p. 21]
Cuminaldehyde	(CLVI)	$C_{10}H_{12}O$	*Cuminum cyminum* L.; *Pectis papposa; Artemisia annua; Eucalyptus* spp.	b.p. 236–237°	[(*5*), p. 156]
Cuminyl alcohol	(CLIII)	$C_{10}H_{14}O$	*Lavandula officinalis* Chaix.	b.p. 248–249°	[(*5*), p. 102]
Cyclolavendulol	(CXXX)		*Lavandula officinalis* Chaix.		(*16*)
p-Cymene	(CLI)	$C_{10}H_{14}$	*Origanum vulgare* L.; (Labiatae Myrtaceae, Umbelliferae, Pinaceae)	b.p. 176–177°	[(*5*), p. 25]
Dehydrogeranic acid	(XXXVII)	$C_{10}H_{14}O_2$	*Callitropsis araucarioides* Compt.; *Callitris glauca*	m.p. 185–186°	[(*5*), p. 311]
d-Dihydrocarveol	(LXV)	$C_{10}H_{18}O$	*Carum carvi*	$[\alpha]_D$ +33.5°; b.p. 107°	[(*1*), Vol. I, p. 280]
(−)-Dihydrocarvone	(LXXXIX)	$C_{10}H_{16}O$	*Carum carvi* L.; *Anethum sowa*	$[\alpha]_D^{20}$ −18°; b.p. 220–222°	[(*1*), Vol. I, p. 350; (*5*), p. 214]

Dihydroxythymoquinone	(CLX)	$C_{10}H_{12}O_4$	*Monarda fistulosa* L.	—	[(*2*), Vol. III, p. 528]
2,6-Dimethyloct-7-en-4-one	(XXXI)	$C_{10}H_{18}O$	*Tagetes glandulifera* Schr.	$[\alpha]_D$ +1.5°; b.p. 185–186°	[(*4*), p. 48]
Diosphenol	(XCV)	$C_{10}H_{16}O_2$	*Barosma* spp.	m.p. 83–84°	[(*1*), Vol. I, p. 413]
Dipentene	(XLVIII)	$C_{10}H_{16}$	Turpentine (Pinaceae, Rutaceae, Myrtaceae, Labiatae)	b.p. 177–178°	[(*1*), Vol. I, p. 143]
β-Dolabrin	(CL)	$C_{10}H_{10}O_2$	*Thujopsis dolabrata*	m.p. 58–59°	(*17*)
Elsholtzia ketone	(XLII)	$C_{10}H_{14}O_2$	*Elsholtzia cristata* Willd.	b.p. 210°	[(*5*), p. 704]
Eucarvone	(CXLV)	$C_{10}H_{14}O$	*Asarum sieboldii* var. *seoulensis* Nakai	b.p. 210–215°	[(*4*), p. 82; (*5*), p. 222]
Evodone	(XCVII)	$C_{10}H_{16}$	*Evodia rutaecarpa* Hook	$[\alpha]_D$ −53.9 ($CHCl_3$); m.p. 73°	[(*5*), p. 704]
Fenchone	(CXXXII)	$C_{10}H_{16}O$	*Foeniculum vulgare* Miller; *Lavandula stoechas; L. burmannii* Benth.; *Thuja occidentalis*	(+): $[\alpha]_D$ +72°; m.p. 5–6°	[(*1*), Vol. II, p. 560; (*4*), p. 146; (*5*), p. 217; (*7*), p. 60]
Fenchyl alcohol	(CXXXIII)	$C_{10}H_{18}O$	*Pinus palustris* Miller	*dl*-α: m.p. 38–39°; *dl*-β: oil, b.p. 201°	[(*1*), Vol. II, p. 552]
Geranial (citral a)	(XXIX)	$C_{10}H_{16}O$	*Cymbopogon flexuosus* Stapf.; geranium oil; lemongrass oil (15%); *Ocimum canum* (17%)	b.p.$_{12}$ 110–112°	[(*4*), p. 46]
Geraniol	(XXIV)	$C_{10}H_{18}O$	*Cymbopogon martini* Stapf. var. *motia*; (Pinaceae, Gramineae, Lauraceae, Rutaceae, Myrtaceae)	b.p.$_{10}$ 110–111°	[(*4*), p. 40]
Geranic acid	(XXVI)	$C_{10}H_{16}O_2$	*Citrus limonum* Burm.; *Eucalyptus dives; Cymbopogon citratus* (=lemongrass oil)	b.p.$_{14}$ 150–152°	[(*5*), p. 309]
Heyderiol	(CLXI)	$C_{22}H_{30}O_4$	*Heyderia decurrens* Torrey	m.p. 62.2–63.2°	(*18*)
4-Hydroxy-β-cyclocitral	(XLV)	$C_{10}H_{16}O_2$	*Chlamydomonas eugametos*	$[\alpha]_D^{20}$ −87° (alcohol)	[(*5*), p. 166]
Hymentherene	(XXI)	$C_{10}H_{16}$	*Hymenatherum tenuifolium* Cass.	—	[(*5*), p. 22]
Iridomyrmecin	(LXXX)	$C_{10}H_{16}O_2$	From the ant *Iridomyrmex humilis*	m.p. 60–61°; $[\alpha]_D$ +210° (4% alcohol)	[(*4*), p. 94]

TABLE XIII (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
Isoartemisia ketone	(CXXIX)	$C_{10}H_{16}O$	*Artemisia annua* L.	b.p. 182–183°	[(*4*), p. 48]
Isoborneol	(CXXIII)	$C_{10}H_{18}O$	Wood turpentine; *Juniperus excelsa*	$[\alpha]_D$ (±)33–34° (alcohol); m.p. 214°	[(*4*), p. 142]
Isolimonene	(IL)	$C_{10}H_{16}$	*Chenopodium ambrosioides* var. *anthelminticum* Gray	b.p. 172–173°	[(*5*), p. 31]
(+)-Isomenthone	(LXXXII)	$C_{10}H_{18}O$	*Mentha pulegium* L.; *M. arvensis; Pelargonium tomentosum*	$[\alpha]_D^{20}$ +93.2°; b.p. 212°	[(*4*), p. 76]
Isopiperitenone	(XCIII)	$C_{10}H_{14}O$	*Mentha pulegium* L.	b.p.$_{2.5}$ 97°	[(*1*), Vol. I, p. 409]
3-Isopropylidene-1-acetylcyclopentene-5	(CXL)	$C_{10}H_{14}O$	*Eucalyptus globulus* L.	$[a]_D$ 0°; b.p.$_{738}$ 225–225.5°	[(*5*), p. 201]
Isopulegol	(LXVII)	$C_{10}H_{18}O$	*Leptospermum liversidgei* var. B.	b.p.$_{12}$ 91°	[(*1*), Vol. I, p. 289]
(+)-Isopulegone	(LXXXVII)	$C_{10}H_{16}O$	*Mentha pulegium* L.; *M. rotundifolia; M. timija; Agastache formosana*	$[\alpha]_D$ +34°; b.p.$_{.5}$ 78°	[(*1*), Vol. I, p. 386]
(−)-Lavandulol	(CXLII)	$C_{10}H_{18}O$	*Lavandula officinalis* Chaix.	$[\alpha]_D^{16}$ −10.2°; b.p.$_{13}$ 94–95°	(*13*)
Libocedrol	(CLXII)	$C_{22}H_{30}O_4$	*Libocedrus decurrens*	m.p. 86.5–88°	[(*5*), p. 91]
3-Libocedroxythymoquinone	(CLXIII)	$C_{32}H_{40}O_6$	*Libocedrus decurrens*	m.p. 154.5–155°	(*19*)
Limonene	(XLVII)	$C_{10}H_{16}$	*Citrus pinensis* L.; *Carum carvi* L.; *Citrus nobilis* var. *deliciosa*	$[\alpha]_D$ (±)124–126°; b.p. 176–178°	[(*1*), Vol. I, p. 143]
Linalool	(XXVI)	$C_{10}H_{18}O$	(+): *Coriandrum sativum* (Pinaceae, Lauraceae, Rutaceae, Labiatae, etc.)	$[\alpha]_D$ (±)19.5°; (+): b.p.$_{12}$ 85–86°	[(*1*), Vol. I, p. 57]
Linalool monoxide	(XXVII)	$C_{10}H_{18}O_2$	*Bursera delpechiana* Poisson.	$[\alpha]_D$ −5.85°; b.p.$_{25}$ 95°	[(*5*), p. 56]
Loganetin	(CXLI)	$C_{11}H_{16}O_5$	*Strychnos nux-vomica*	$[\alpha]_D^{20}$ −23° (alcohol)	[(*5*), p. 388]
Macropone	(CLVII)	$C_{10}H_{12}O_2$	*Eucalyptus cneorifolia*	b.p.$_{.2}$ 108°	[(*5*), p. 157]

p-Menthane	(XLVI)	$C_{10}H_{20}$	*Pinus* spp.; wood turpentine	*cis:* b.p. 168.5° *trans:* b.p. 161°	[(*2*), Vol. VI, p. 295]
p-Menthanediol-(1,4)	(LXXI)	$C_{10}H_{20}O_2$	*Cupressus torulosa*	1-Form: m.p. 136.5–137.5° 2-Form: m.p. 116–117°	[(*1*), Vol. I, p. 304]
p-Menthanol-(8)	(LVI)	$C_{10}H_{20}O$	Pine oil	*trans:* m.p. 35° *cis:* m.p. 25°	[(*1*), Vol. I, p. 253]
p-Menthanol-(4)	(LVII)	$C_{10}H_{20}O$	Wood turpentine	b.p.$_{13}$ 88–89°	[(*5*), p. 121]
Menthofuran	(XCVI)	$C_{10}H_{14}O$	*Mentha piperita* L.	$[\alpha]_D^{25}$ +92.5°	[(*4*), p. 74; (*5*), p. 702]
l-Menthol	(LVIII)	$C_{10}H_{20}O$	*Mentha piperita vulgaris* S. (Labiatae, Geraniaceae, etc.)	$[\alpha]_D$ −50° (alcohol) m.p. 43°	[(*4*), p. 81]
Menthone	(LXXXI)	$C_{10}H_{18}O$	(−): *Mentha* spp. *M. pulegium* L.; *M. arvensis; Bystropogon origanifolius; B. mollis*	$[\alpha]_D$ −28.5°; b.p. 210°	[(*4*), p. 76]
Mesogentiogenin	(XXXVIII)	$C_{10}H_{10}O_4$	Aglycon of gentiopicrin	Oil	[(*5*), p. 449]
1-Methyl-4-acetylcyclohexene-(6)	(CLXXII)	$C_9H_{14}O$	*Cedrus atlantica*	b.p. 205–206°	[(*5*), p. 203]
Methylheptenone	(CLXIV)	$C_8H_{14}O$	Lemongrass oil; *Zingiber officinale* Rosc.; geranium and citronella oils	b.p. 173–174°	[(*5*), p. 173]
1-Methyl-4-isopropenylbenzene	(CLII)	$C_{10}H_{12}$	*Cannabis indica* Lam.	b.p. 186–189°	[(*5*), p. 26]
8-Methylnonen-(2)-al-(1)	(CXXVII)	$C_{10}H_{18}O$	*Coriandrum sativum*	—	[(*5*), p. 150]
β-Myrcene	(XX)	$C_{10}H_{16}$	*Myrcia acris* D. C.; *Rhus cotinus* (50%); *Salvia sclarea; Dacrydium* spp.	b.p.$_{12}$ 55–56°	[(*1*), Vol. I, p. 12]
Myrcenone	(XXXIV)	$C_{10}H_{14}O$	*Lippia asperifolia* Rich.	b.p.$_{2\cdot1}$ 63–64°	[(*5*), p. 175]
Myrtenal	(CXVII)	$C_{10}H_{14}O$	*Hernandia peltata* Meissn.; *Eucalyptus globulus; Calytrix tetragona*	$[\alpha]_D$ +13 t. +17°; b.p.$_{12\cdot5}$ 91.5°	[(*4*), p. 124]
Myrtenic acid	(CXXI)	$C_{10}H_{14}O_2$	*Chamaecyparis formosensis*	$[\alpha]_D$ +51.1°; m.p. 53–54°	[(*5*), p. 398]

TABLE XIII (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
Myrtenol	(CXV)	$C_{10}H_{16}O$	*Myrtus communis* L.; *Chamaecyparis formosensis; Darwinia grandiflora*	(+): $[\alpha]_D^{20}$ +46.5°; b.p. 222–224°	[(*1*), Vol. I, p. 68]
l-Neodihydrocarveol	(LXVI)	$C_{10}H_{18}O$	*Carum carvi* L.	$[\alpha]_D$ −32.5° to −33.2°; b.p. 214–215°	[(*1*), Vol. I, p. 283]
d-Neomenthol	(LIX)	$C_{10}H_{20}O$	*Mentha arvensis* L.	$[\alpha]_D$ +19.6–20.7°	[(*4*), p. 76; (*5*), p. 121]
Nepetalactone	(LXXVIII)	$C_{10}H_{14}O_2$	*Nepeta cataria* L.	b.p.$_{13}$ 129–130°	[(*5*), p. 452; (*4*), p. 92]
Nepetalic acid	(LXXIX)	$C_{10}H_{16}O_3$	*Nepeta cataria* L.	$[\alpha]_D^{23}$ +48.1°; m.p. 74–75°	[(*4*), p. 92]
Neral (=Citral b)	(XXX)	$C_{10}H_{16}O$	*Cymbopogon flexuosus* Stapf.; Lemongrass oil	b.p.$_{12}$ 110–112°	[(*1*), Vol. I, p. 83]
Nerol	(XXV)	$C_{10}H_{18}O$	*Helichrysum angustifolium* D. G. (Pinaceae, Gramineae, Rutaceae, Labiatae, Compositae)	b.p.$_{25}$ 125°	[(*4*), p. 42]
Nortricycloekasantalal	(CXXXVI)	$C_{11}H_{16}O$	*Santalum album* L.	$[\alpha]_D$ −38.8°; b.p. 222–224°	[(*7*), p. 77]
β-Ocimene	(XIX)	$C_{10}H_{16}$	*Ocimum basilicum* L.; *Evodea rutaecarpa; Salvia sclarea*	b.p.$_{19}$ 72–74°	[(*4*), Vol. II, p. 38]
Ocimenone	(XXXIII)	$C_{10}H_{14}O$	*Lippia asperifolia* Rich.	b.p.$_{2\cdot1}$ 63–64°	[(*5*), p. 175]
(+)-Orthodene	(CXXXVII)	$C_{10}H_{16}$	*Orthodon formosanum* Kudo	$[\alpha]_D^{29}$ +32.6°; b.p. 168–170°	[(*5*), p. 36]
Perillaldehyde	(LXXVI)	$C_{10}H_{14}O$	*Perilla nankinensis* Decne (+): *Siler trilobum* Scop.	(+): $[\alpha]_D$ +136–139° (−): $[\alpha]_D$ −146°	[(*4*), Vol. II, p. 86]
Perilla ketone	(XLI)	$C_{10}H_{14}O_2$	*Perilla frutescens* Brit.	b.p.$_{20}$ 118–119°	[(*5*), p. 704]
Perillene	(XXXIX)	$C_{10}H_{14}O$	*Perilla citriodora* Makino	b.p. 185–186°	[(*5*), p. 701]

Perillyl alcohol	(LXX)	$C_{10}H_{16}O$	*Andropogon schoenanthus* L. *Cymbopogon* spp.	b.p.$_5$ 94–96°	[(*4*), p. 86]
Phellandral	(LXXV)	$C_{10}H_{16}O$	*Phellandricum aquaticum* L.; *Eucalyptus* spp. (Myrtaceae, Umbelliferae)	(+): $[\alpha]_D$ +123° (−): $[\alpha]_D$ −133.4 to −138.9°	[(*1*), Vol. I, p. 308]
α-Phellandrene	(LIII)	$C_{10}H_{16}$	*Cinnamomum zeylanicum* Nees.; *Eucalyptus dives.*	$[\alpha]_D$ max. −177.4°; b.p. 175–176°	[(*1*), Vol. I, p. 193]
β-Phellandrene	(LIV)	$C_{10}H_{16}$	*Illicium verum* Hooker; Oil of star anise (Pinaceae, Myrtaceae, Lauraceae, Compositae, Umbelliferae)	$[\alpha]_D$ (±)52°; b.p. 171–172°	[(*1*), Vol. I, p. 204]
Phellandric acid	(LXXVII)	$C_{10}H_{16}O_2$	*Bursera microphylla*	m.p. 144–145°	[(*5*), p. 392]
α-Pinene	(CX)	$C_{10}H_{16}$	*Pinus sylvestris; P. maritima; l-α-*Pinene in 30 phanerogam families (Pinaceae, Myrtaceae, Rutaceae, Umbelliferae, Labiatae, etc.)	(+): $[\alpha]_D$ +48.3 to +51.5 (−): $[\alpha]_D$ −43.4 to −51.5; b.p. 155–157°	[(*1*), Vol. II, p. 105; (*5*), p. 36]
β-Pinene	(CXI)	$C_{10}H_{16}$	*Pinus* spp.; In 100 species of Pinaceae, Lauraceae, Rutaceae, Myrtaceae, Labiatae, etc.	(+): $[\alpha]_D$ +20.8 (−): $[\alpha]_D$ −21.5°; b.p. 163–166°	[(*1*), Vol. II, p. 191; (*5*), p. 37]
(−)-Pinocampheol	(CXII)	$C_{10}H_{18}$	*Hyssopus officinalis* L.	$[\alpha]_D^{21}$ −55.3°; m.p. 67–68°	[(*4*), p. 124; (*5*), p-128]
Pinocamphone	(CXVIII)	$C_{10}H_{16}O$	(−): *Hyssopus officinalis* L.	*cis:* α_D (±)18°; *trans:* α_D (±)22.5°	[(*5*), p. 219]
(−)-Pinocarveol	(CXIII)	$C_{10}H_{16}O$	*Eucalyptus globulus* Lab.	$[\alpha]_D$ −61.7; m.p. 5–7°	[(*5*), p. 133]
(−)-Pinocarvone	(CXIX)	$C_{10}H_{14}O$	*Eucalyptus globulus* Lab. *Chenopodium ambrosioides*	$[\alpha]_D$ −68.5°	[(*5*), p. 220]
Piperitenone	(XCI)	$C_{10}H_{14}O$	*Mentha pulegium* L. var. *villosa* Benth.	b.p.$_{1.8}$ 92°	[(*5*), p. 214]
Piperitenone oxide	(XCII)	$C_{10}H_{14}O_2$	*Mentha rotundifolia*	$[\alpha]_D$ +150.6°; m.p. 25.6–26°	[(*5*), p. 215]

TABLE XIII (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
Piperitol	(LX)	$C_{10}H_{18}O$	*Eucalyptus radiata* Sieb.	(−): $[\alpha]_D$ −34.1° (+): $[\alpha]_D$ +40.2°	[(*1*), Vol. I, p. 286]
Piperitone	(LXXXIV)	$C_{10}H_{16}O$	*Mentha arvensis* var. *piperasceus* Holm.; *Eucalyptus piperita; Andropogon iwarancusa* Jones	(+): $[\alpha]_D^{15}$ +62.5° (−): $[\alpha]_D^{20}$ −67.8°	[(*1*), Vol. I, p. 359]
Piperitone oxide	(LXXXV)	$C_{10}H_{16}O_2$	*Mentha sylvestris*	m.p. 14.5–15.5°; $[\alpha]_D^{22}$ −177°	[(*5*), p. 212]
Pulegone	(LXXXVI)	$C_{10}H_{16}O$	*Mentha pulegium* L.; *Origanum dictamnus* L.; *Hedeoma pulegioides*	$[\alpha]_D^{20}$ +23.7 to +27.8°; b.p. 222–224°	[(*4*), p. 176] [(*1*), Vol. I, p. 370]
Sabinene	(IC)	$C_{10}H_{16}$	*Juniperus sabina* L.; *Thuja occidentalis; Piper cubeba* L.; *Chamaecyparis* spp.	$[\alpha]_D$ +89.1°; b.p. 163–165°	[(*4*), p. 102]
Sabinol	(CII)	$C_{10}H_{16}O$	*Juniperus sabina* L.; *Thujopsis dolabrata* Sieb. et Zucc.	b.p.$_{11}$ 90°	[(*4*), p. 104; (*1*), Vol. II, p. 28]
Safranal	(XLIV)	$C_{10}H_{14}O$	*Crocus sativus* L.	b.p.$_{12}$ 85–87°	[(*1*), Vol. I, p. 120]
Salvene	(XCVIII)	$C_{10}H_{18}$	*Salvia officinalis*	b.p. 142–145°	[(*5*), p. 39]
Santene	(CLXVIII)	C_9H_{14}	*Abies excelsa; A. sibirica* Ledeb.; *Santalum album* L.	b.p. 139–142°	[(*1*), Vol. II, p. 243]
Santenol	(CLXVI)	$C_9H_{16}O$	East Indian sandalwood oils	m.p. 58–62°	[(*1*), Vol. II, p. 251]
Santenone	(CLXVII)	$C_{10}H_{16}O$	Fennel oil; East Indian sandalwood oil	$[\alpha]_D$ −4.7°; m.p. 48–52°	[(*1*), Vol. II, p. 257]
β-Santolinenone	(CXXXVIII)	$C_{10}H_{16}O$	*Santolina chamaecyparis* L.	—	[(*1*), Vol. I, p. 393]
α-Santolinenone	(XC)	$C_{10}H_{16}O$	*Santolina chamaecyparis* L.	—	[(*1*), Vol. I, p. 390]
Shonanic acid	(CXXXI)	$C_{10}H_{14}O_2$	*Librocedrus formosana*	$[\alpha]_D^{18}$ −0.75° (alcohol); m.p. 40–41°	[(*5*), p. 393]
Tagetone	(XXXII)	$C_{10}H_{16}O$	*Tagetes glandulifera* Schr.	b.p. 205–210°	[(*1*), Vol. I, p. 102]

Teresantalol	(CXXV)	$C_{10}H_{16}O$	*Santalum album*	$[\alpha]_D$ +11.6° (alcohol); m.p. 112–114°	[(*1*), Vol. II, p. 264]
Teresantalic acid	(CXXXV)	$C_{10}H_{14}O_2$	*Santalum album* L.	$[\alpha]_D^{20}$ −76.6° (benzol); m.p. 157°	[(*1*), Vol. II, p. 264]
α-Terpinene	(L)	$C_{10}H_{16}$	*Juniperus sabina* L.	b.p.$_{15}$ 62°	[(*4*), p. 61; (*5*), p. 28]
β-Terpinene	(LI)	$C_{10}H_{16}$	*Pittosporum tenuifolium*	b.p. 176°	[(*4*), p. 61; (*5*), p. 30]
γ-Terpinene	(LII)	$C_{10}H_{16}$	*Coriandrum sativum; Crithmum maritimum* L.; *Eucalyptus* spp.	b.p.$_{18}$ 72.5°	[(*4*), p. 61; (*5*), p. 28]
Terpinenol-(1)	(LXIV)	$C_{10}H_{18}O$	Turpentine	b.p. 208–210°	[(*1*), Vol. I, p. 279]
Terpineol-(4)	(LXI)	$C_{10}H_{18}O$	*Cupressus sempervirens* L.	$[\alpha]_D$ (±)24.5–25°; b.p. 208–212°	[(*1*), Vol. I, p. 275]
α-Terpineol	(LXII)	$C_{10}H_{18}O$	Pine oil (Pinaceae, Rutaceae, Umbelliferae, Labiatae, Lauraceae, Myrtaceae)	(+): $[\alpha]_D$ +106°; m.p. 37–39° (−): $[\alpha]_D$ −117.5°; m.p. 37–38°	[(*1*), Vol. I, p. 256]
γ-Terpineol	(LXIII)	$C_{10}H_{18}O$	*Cupressus torulosa* Don.	m.p. 69–70°	[(*1*), Vol. I, p. 271]
Terpinolene	(LV)	$C_{10}H_{16}$	*Canarium luzonicum* Gray.; *Juniperus occidentalis; Ocimum canum* Sims.	b.p.$_{14}$ 72–73°	[(*1*), Vol. I, p. 165]
α-Thujaplicin	(CXLVII)	$C_{10}H_{12}O_2$	*Thuja plicata; T. occidentalis; Thujopsis dolobrata*	m.p. 34°	[(*5*), p. 224]
β-Thujaplicin	(CXLVIII)	$C_{10}H_{12}O_2$	*Chamaecyparis* spp.; *Thuja plicata; T. occidentalis; Chamaecyparis* spp. *Thujopsis dolabrata; Libocedrus decurrens*	m.p. 52–53°	[(*5*), p. 225]
γ-Thujaplicin	(CIL)	$C_{10}H_{12}O_2$	*Thuja plicata; T. occidentalis; Libocedrus decurrens; Chamaecyparis thyoides*	m.p. 79–80°	[(*5*), p. 225]

TABLE XIII (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
d-α-Thujene	(C)	$C_{10}H_{16}$	*Boswellia serrata* Roxb.; *Chamaecyparis obtusa* Sieb. et Zucc.; *Thuja occidentalis; Eucalyptus dives*	$[\alpha]_D$ +38.8°; b.p. 151–153°	[(*1*), Vol. II, p. 10]
Thujic acid	(CXLVI)	$C_{10}H_{12}O_2$	*Thuja plicata* D. Don.	m.p. 88–89°	[(*5*), p. 399]
Thujone	(CIII)	$C_{10}H_{16}O$	*Thuja occidentalis* Hort.; *Salvia officinalis; Tanacetum* spp.	α: $[\alpha]_D$ −10 to −20° β: $[\alpha]_D$ +75°	[(*1*), Vol. II, p. 32; (*5*), p. 219]
Thujyl alcohol	(CI)	$C_{10}H_{18}O$	*Artemisia absinthum* L. (Pinaceae, Gramineae, Rutaceae, Compositae)	Oil	[(*1*), Vol. II, p. 23]
Thymohydroquinone	(CLVIII)	$C_{10}H_{14}O_2$	*Carum roxburghianum* Benth.	m.p. 139–142°	[(*5*), p. 90]
Thymol	(CLV)	$C_{10}H_{14}O$	*Thymus vulgaris* L.	m.p. 50–51.5°	[(*5*), p. 77]
Thymoquinone	(CLIX)	$C_{10}H_{12}O_2$	*Libocedrus decurrens* Torrey	m.p. 45.5–48°	[(*5*), p. 480]
α-*p*-Tolylethyl alcohol	(CLXXIV).	$C_9H_{12}O$	*Curcuma domestica*	b.p.$_{19}$ 120°	[(*5*), p. 102]
1,1,3-Trimethylcyclohexanone-(2)	(CLXV)	$C_9H_{16}O$	*Citrus* spp.	b.p.$_{10}$ 66–67°	[(*5*), p. 202]
1,1,3-Trimethylcyclopentanone-(4)	(CLXIX)	$C_8H_{14}O$	*Mentha pulegium*	b.p. 159.5–160.5°	[(*5*), p. 199]
Umbellulone	(CIV)	$C_{10}H_{14}O$	*Umbellularia californica* Nutt.	$[\alpha]_D$ −36 to −37°; b.p. 219–220°	[(*1*), Vol. II, p. 52]
Verbenalol	(CXLIV)	$C_{11}H_{14}O_5$	Aglycon of verbenalin	$[\alpha]_D^{24}$ −20 to −21°; m.p. 133–135°	[(*5*), p. 453]
Verbenol	(CXIV)	$C_{10}H_{16}O$	*Boswellia carteri* Birdw.	*cis*: $[\alpha]_D$ +65.3° *trans*: $[\alpha]_D$ +169.5°	[(*1*), Vol. II, p. 214]
Verbenone	(CXX)	$C_{10}H_{14}O$	*Verbena triphylla* L.	$[\alpha]_D$ +273.3°; m.p. 9.8°	[(*1*), Vol. II, p. 232]

TABLE XIV

Sesquiterpene Compounds: Occurrence, Formulas, and Physical Constants

Compound	Number	Formula	Source	Physical Constants	References
Acorone	(CCXXIX)	$C_{15}H_{24}O_2$	Sweet flag oil	m.p. 101°	[(*4*), p. 310]
Agarol	(CCXXXVI)		Agarwood oil	—	(*20*)
Ambrosin	(CCXCI)	$C_{15}H_{18}O_3$	*Ambrosia maritima* L.	m.p. 146°	(*21*, *22*, *146*)
Arborescin	(CCLXXXIX)	$C_{15}H_{20}O_3$	*Artemisia arborescens*	$[\alpha]_D^{20}$ +63° (alcohol); m.p. 145°	[(*4*), p. 272]
Arctiopicrin	(CCLXVIII)	$C_{19}H_{28}O_6$	*Arctium minus* Bernh.	$[\alpha]_D$ +133°; m.p. 115°	(*23*)
Aristolactone	(CCLXIV)	$C_{15}H_{20}O_2$	*Aristolochia reticulata*	m.p. 110–111°	(*24*)
Aristolone	(CCCIX)	$C_{15}H_{24}O$	*Aristolochia debilis*	$[\alpha]_D^{12}$ −339.4;° m.p. 101°	[(*5*), p. 221]
Aromadendrene	(CCLXXX)	$C_{15}H_{24}$	*Eucalyptus globulus* L.; *Pinus lambertiana* Dougl.; *Dacrydium biforme; Leptospermum flavescens*	b.p. 123–125°	[(*1*), Vol. III, p. 71] [(*4*), p. 246; (*25*) (*5*), p. 770]
Artabasin	(CCLXXXVI)	$C_{15}H_{20}O_3$	*Artemisia absinthum*	$[\alpha]_D$ −49°; m.p. 133–135°	[(*4*), p. 264]
Artemesin	(CCXLIV)	$C_{15}H_{18}O_4$	*Artemisia maritima* Linn.	$[\alpha]_D$ −84.3° (alcohol); m.p. 203°	[(*4*), p. 228] [(*1*), Vol. III, p. 312]
Atlantolactone	(CCXLII)	$C_{15}H_{20}O_2$	*Inula helenium* L.	m.p. 74–76°	[(*1*), Vol. III, p. 240]
α-Atlantone	(CCV)	$C_{15}H_{22}O$	*Cedrus atlantica* Manet	b.p. 121–122°	[(*5*), p. 752]
γ-Atlantone	(CCVI)	$C_{15}H_{22}O$	*Cedrus altantica* Manet	b.p.$_1$ 121–122°	[(*4*), p. 202; (*5*), p. 752]
Balduilin	(CCC)	$C_{17}H_{20}O_5$	*Balduina uniflora* Nutt.	$[\alpha]_D^{24}$ +57°; m.p. 231–232°	(*26*)
α-Betulenol	(CCLX)	$C_{15}H_{24}O$	*Betula alba* L.; *B. lenta* L.	$[\alpha]_D$ −19.5°; b.p.$_{20}$ 154–156°	(*27*)

TABLE XIV (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
β-Betulenol	(CCLXI)	$C_{15}H_{24}O$	*Betula alba* L.; *B. lenta* L.	$[\alpha]_D$ −36°; b.p.$_{20}$ 155–157°	(*27*)
Bisabolene	(CXCVIII)	$C_{15}H_{24}$	*Commifora erythraea* Engl.; *Abies sibirica* Ledeb.; *Cinnamomum camphora* Nees; *Ocimum* spp.	$[\alpha]_D$ −41.3°; b.p. 262°	[(*1*), Vol. III, p. 9; (*4*), p. 192; (5), p. 750]
Bisabolol	(CIC)	$C_{15}H_{26}O$	*Matricaria chamomilla Populus balsamifera*	$[\alpha]_D$ −55.7°; b.p.$_{12}$ 155–157°	[(*4*), p. 192]
α-Cadinene	(CCXI)	$C_{15}H_{24}$	*Piper cubeba* L.	—	[(*4*), p. 204]
γ-Cadinene	(CCXII)	$C_{15}H_{24}$	Ylang-ylang	$[\alpha]_D$ +3.8°	(*28*)
ε-Cadinene	(CCXIII)	$C_{15}H_{24}$	Oil from Ylang-ylang	—	[(*4*), p. 204]
α-Cadinol	(CCXXI)	$C_{15}H_{26}O$	Citronella oil; *Cedrela toona* Roxb.	m.p. 77.5–78°	[(*5*), p. 754; (*29*)]
δ-Cadinol	(CCXXII)	$C_{15}H_{26}O$	*Juniperus communis; Chamaecyparis lawsoniana*	m.p. 139–140°	(*30*)
Calamenediol	(CCXXV)	$C_{15}H_{26}O_2$	Calamus oil	$[\alpha]_D$ −4.4; m.p. 168–169°	[(*5*), p. 757]
Calamenene	(CCXX)	$C_{15}H_{22}$	*Acorus calamus* L.	b.p.$_{15}$ 136–143°	(*31*)
Calythrone	(CCCXXVII	$C_{12}H_{16}O_3$	*Jasminum grandiflorum* L.	b.p.$_{15}$ 145–147°	[(*5*), p. 201]
Carissone	(CCXXXIX)	$C_{15}H_{24}O_2$	*Carissa lanceolata* R. Br.	m.p. 78–79°; $[\alpha]_D$ +136.6° ($CHCl_3$)	[(*4*), p. 216]
Carotol	(CCCXIV)	$C_{15}H_{26}O$	*Daucus carota* L.	$[\alpha]_D$ +28° (alcohol); b.p.$_{1\cdot5}$ 109°	(*32*, *33*)
Carpesia lactone	(CCXC)	$C_{15}H_{20}O_3$	*Carpesium abrotanoides*	b.p.$_5$ 200–220°	[(*4*), p. 276; (*5*), p. 451]
β-Caryophyllene	(CCLVIII) (CCLIX)	$C_{15}H_{24}$	*Eugenia caryophyllata* Thumb.	$[\alpha]_D^{20}$ −9°	[(*1*), Vol. V, p. 517; (*4*), p. 292]
Cedrene	(CCXXVII)	$C_{15}H_{24}$	*Juniperus* spp.; *J. excelsa; J. occidentalis; Salvia sclarea* L.; *Pterodon pubescens* Benth.	$[\alpha]_D$ −55 to −71°; b.p.$_{12}$ 124–126°	[(*1*), p. Vol. V, p. 534; (*4*), p. 278]

Cedrol	(CCXXVIII)	$C_{15}H_{26}O$	*Juniperus* spp.; *Biota orientalis; Boldea fragrans* Juss.	$[\alpha]_D$ +9.3 to +10.5°; m.p. 84–87°	[(*1*), Vol. V, p. 534; (*4*), p. 285]
α-Chigadmarene	(CCLXXII)	$C_{15}H_{24}$	*Lansium annamalayanum*	$[\alpha]_D^{28}$ −150°; b.p. 117–119°	[(*1*), Vol. V, p. 548; (*4*), p. 258]
Cnicin	(CCLXIX)	$C_{20}H_{28}O_7$	*Cnicus benedictus* L.	m.p. 143°	(*34*, *140*)
Copaene	(CCXXVI)	$C_{15}H_{24}$	*Oxystigma mannii* Harms (copaiva resin); *Cedrela toona* Roxb.; *Sindora wallichii*	b.p.$_{10}$ 114–115°	[(*1*), Vol. III, p. 88]
Coriamyrtione	(CCCXXI)	$C_{15}H_{18}O_5$	*Coriaria japonica*	—	[(*5*), p. 1111]
Costunolide	(CCLXV)	$C_{15}H_{20}O_2$	*Artemisia balchanorum*	$[\alpha]_D^{20}$ +128° ($CHCl_3$); m.p. 106–107°	(*35*)
l-α-Curcumene	(CCI)	$C_{15}H_{22}$	*Curcuma aromatica* Salisb.	$[\alpha]_D$ −22.9°; b.p.$_{17}$ 137°	[(*4*), p. 198]
β-Curcumene	(CCII)	$C_{15}H_{22}$	*Curcuma aromatica* Salisb.; *Nectandra elaiophora*	b.p.$_{19}$ 142°	[(*4*), p. 198; (*5*), p. 749]
γ-Curcumene	(CCIII)	$C_{15}H_{22}$	*Libocedrus bidwillii* Hook	$[\alpha]_D$ +31.8°; b.p.$_3$ 94°	[(*1*), Vol. I, p. 514]
Cyclocolorenone	(CCLXXXIV)	$C_{15}H_{22}O$	*Pseudowintera colorata*	$[\alpha]_D$ −400° (alcohol); b.p.$_5$ 136–138°	(*36*)
α-Cyperone	(CCXXXVII)	$C_{15}H_{22}O$	*Cyperus rotundus* L.; *C. scariosus* R. Br.	b.p.$_{20}$ 177°	[(*4*), p. 214]
Damarin	(CCXV)	$C_{15}H_{24}$	Dammar resin; *Canarium strictum* Roxb.	b.p.$_{12}$ 114–132°; $[\alpha]_D$ −14°	(*37*)
Dammar diketone	(CCCXVII)	$C_{14}H_{20}O_2$	Dammar resin	b.p.$_2$ 125-136°	(*37*)
Damsin	(CCXCII)	$C_{15}H_{20}O_3$	*Ambrosia maritima* L.	m.p. 102°	(*22*, *146*)
Daucol	(CCCXV)	$C_{15}H_{26}O_2$	*Daucus carota* L.	—	(*33*)
Dehydrocostus lactone	(CCLXXXV)	$C_{15}H_{18}O_2$	*Saussurea lappa* Clarke	$[\alpha]_D^{20}$ −21.9° (alcohol); m.p. 61°	(*38a*)

TABLE XIV (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
Dendrolasin	(CXCIV)	$C_{15}H_{22}O$	From the ant *Lasius* (*Dendrolasius*) *fuliginosus* Latr.	b.p.$_{16}$ 148–150°; $[\alpha]_D$ 0°	(*38b*)
Drimenol	(CCLIV)	$C_{15}H_{26}O$	*Drimus winteri* Forst.	$[\alpha]_D$ −18°; m.p. 97–98°	(*39*, *40*)
α-Elemenone	(CCCXI)	$C_{15}H_{22}O$	*Geranium macrorrhizum* L.	m.p.$_{15}$ 151–154°	(*41*)
Elemol	(CCCX)	$C_{15}H_{26}O$	*Canarium luzonicum* Gray Java citronella oil	$[\alpha]_D$ −9.6° (benzene); m.p. 47–54°	[(*4*), p. 210]
Eremophilone	(CCCVI)	$C_{15}H_{22}O$	*Eremophila mitchelli* Benth.	$[\alpha]_D$ −171.6° (alcohol)	(*42*)
α-Eudesmol	(CCXXXI)	$C_{15}H_{26}O$	*Eucalyptus macarthuri* D. et Maid.; other *E.* spp.; *Cryptomeria japonica* Don. in mixture of α- and β-eudesmol	—	[(*1*), Vol. V, p. 561; (*4*), p. 210]
β-Eudesmol	(CCXXXII)	$C_{15}H_{26}O$		m.p. 82–83°	[(*1*), Vol. V, p. 561; (*4*), p. 210; (*5*), p. 758]
d-Eupatene	(CCXVIII)	$C_{15}H_{24}$	*Eupatorium odoratum*	$[\alpha]_D$ +16.25 b.p.$_2$ 92–94°	(*43*)
Farnesal	(CXCIII)	$C_{15}H_{24}O$	Oil of lemongrass	b.p.$_{2\cdot5}$ 135–140°	[(*2*), Vol. IV, p. 41]
Farnesene	—	$C_{15}H_{24}$	*Humulus lupulus*	—	[(*5*), p. 24]
Farnesiferol A	(CCLV)	$C_{24}H_{30}O_4$	*Ferula asafoetida*	m.p. 155–155.5°	(*44*)
Farnesiferol B	(CCLVI)	$C_{24}H_{30}O_4$	*Ferula asafoetida*	m.p. 112.5–113.5°	(*44*)
Farnesol	(CXCI)	$C_{15}H_{26}O$	Australian sandalwood oil (Gramineae, Leguminosae, Rutaceae)	b.p.$_{10}$ 160°	[(*1*), Vol. III, p. 115; (*4*), p. 184]
Geigerin	(CCXCIII)	$C_{15}H_{20}O_4$	*Geigera* spp.	m.p. 192°	(*46*)
Geigerenin	(CCLXXXVIII)	$C_{15}O_{22}O_4$	*Geigera asperia* Harv.	$[\alpha]_D^{29}$ −10.7° (alcohol); m.p. 202–203°	(*45*)
Geijerene	(CCCXIII)	$C_{12}H_{18}$	*Geijera parviflora* Lindley	$[\alpha]_D$ 0°	(*47*)
Germacrone	(CCXL) and (CCLXIII)	$C_{15}H_{22}O$	*Geranium macrorrhizum* L.	$[\alpha]_D$ 0°; m.p. 55–56°	(*48*, *49*)

Globulol	(CCLXXXII)	$C_{15}H_{26}O$	*Eucalyptus globulus* L.	$[\alpha]_D$ −35.3° ($CHCl_3$); m.p. 88.5°	(*50, 51*)
Grifolin	(CCCXXIX)	$C_{16}H_{28}O_2$	*Grifola confluens*	m.p. 40–41°	[(*5*), p. 60]
Guaiazulene	(CLXXXIV)	$C_{15}H_{18}$	*Eucalyptus globulus;* blue camphor oil; callitris oil; *Dipterocarpus* spp.	m.p. 31.5° blue	[(*4*), p. 182; (*5*), p. 780]
Guaiazulenic acid	(CCLXX)	$C_{15}H_{16}O_2$	*Achillea millefolium* L.	m.p. 86–88°	(*52*)
Guaiol	(CCLXXIII)	$C_{15}H_{26}O$	*Bulnesia sarmienti* Lor.; *Eucalyptus maculata*	$[\alpha]_D^{20}$ −30°; m.p. 91°	[(*4*), p. 244]
α-Gurjunene	(CCLXXIX)	$C_{15}H_{24}$	*Dipterocarpus* spp. (gurjun resin); *Hypericum perforatum* L.	α: $[\alpha]_D$ −179.5° β: $[\alpha]_D$ +74.5°	[(*5*), p. 766; (*53, 54*)]
Helenalin	(CCIC)	$C_{15}H_{18}O_4$	*Helenium* spp.; *H. autumnale; H. macrocephalum; H. quadridentalum*	$[\alpha]_D^{20}$ −102° (alcohol); m.p. 169–172°	[(*1*), p. 593; (*4*), p. 270; (*5*), p. 777]
Humulene	(CCLVII)	$C_{15}H_{24}$	*Humulus lupulus* L.; *Pinus maritima; Salvia grandiflora; Artemisia annua; Orthodon* spp.	$[\alpha]_D$ 0°	[(*4*), p. 302; (*145*); (*5*), p. 778]
Hydroxydihydro-eremophilone	(CCCVIII)	$C_{25}H_{24}O_2$	*Eremophila mitchelli* Benth.	$[\alpha]_D$ +90.6° ($CHCl_3$); m.p. 102–103°	[(*4*), p. 236
Hydroxyeremophilone	(CCCVII)	$C_{15}H_{22}O_2$	*Eremophila mitchelli* Benth.	$[\alpha]_D$ −171.6° (alcohol); m.p. 42–43°	[(*4*), p. 236; (*42*)]
Ipomeamarone (*cis*)	(CXCVI)	$C_{15}H_{22}O_3$	*Solanum tuberosum* L.	$[\alpha]_D^{25}$ +28°; b.p.$_4$ 130–135°	[(*4*), p. 188]
Iresin	(CCLIII)	$C_{15}H_{22}O_4$	*Iresine celosioides*	$[\alpha]_D^{28}$ +21° ($CHCl_3$); m.p. 140–142°	[(*4*), p. 308; (*55*)]
α-Irone	(CCCXXIV)	$C_{14}H_{22}O$	*Iris* spp.; *I. germanica; I. pallida* Lam.	$[\alpha]_D$ +70° (alcohol)	[(*4*), p. 90; (*5*) p. 204]
β-Irone	(CCCXXV)	$C_{14}H_{22}O$	*Iris germanica*	$[\alpha]_D$ +42–44° (alcohol)	[(*4*), p. 90; (*5*), p. 205]

TABLE XIV (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
γ-Irone	(CCCXXVI)	$C_{14}H_{22}O$	*Iris germanica*	$[\alpha]_D$ +8°	[(*4*), p. 90; (*5*), p. 206]
Isoatlantolactone	(CCXLIII)	$C_{15}H_{20}O_2$	*Inula helenium* L.	m.p. 115°	[(*1*), Vol. III, p. 240]
Isohelenalin	(CCCI)	$C_{15}H_{18}O_4$	*Helenium* spp.	m.p. 260–262°	[(*4*), p. 272]
Isotenulin	(CCXCV)	$C_{17}H_{22}O_5$	*Helenium tenuifolium*	$[\alpha]_D$ +6° (alcohol); m.p. 160–161°	[(*5*), p. 776]
d-α-Ionone	(CCCXXII)	$C_{13}H_{20}O$	*Boronia megastigma; Saussurea lappa* Clarke; *Lawsonia inermis*	$[\alpha]_D^{20}$ +378.7°; b.p.$_{1.9}$ 86–87°	[(*4*), p. 88; (*5*), p. 203]
β-Ionone	(CCCXXIII)	$C_{13}H_{20}O$	*Boronia megastigma; Saussurea lappa* Clarke; *Lawsonia inermis*	b.p.$_{10}$ 127–129°	[(*4*), p. 88; (*5*), p. 203]
Iunenol	(CCXXXIII)	$C_{15}H_{26}O$	*Juniperus communis* L.	m.p. 62.5–63°; b.p.$_1$ 121–122°	(*56*)
Juniper camphor	(CCXXXIV)	$C_{15}H_{26}O$	*Juniperus communis* L.	m.p. 166.5°	(*57*)
Kessoglycol	(CCLXXVII)	$C_{15}H_{26}O_3$	*Valeriana officinalis* var. *angustifolia*	$[\alpha]_D$ −24.4°; m.p. 128°	[(*4*), p. 254; (*5*), p. 774; (*143*)]
α-Kessyl alcohol	(CCLXXVI)	$C_{15}H_{26}O$	*Valeriana officinalis* var. *angustifolia; V. japonica*	$[\alpha]_D^{20}$ −45° (alcohol); m.p. 85°	[(*4*), p. 254; (*5*), p. 774; (*142*, *143*)]
Kijanene	(CCXIX)	$C_{15}H_{24}$	*Cryptomeria japonica*	$[\alpha]_D$ −22.4°; b.p.$_{4.5}$ 108–114°	[(*5*), p. 755]
Kijanol	(CCXXIII)	$C_{15}H_{26}O$	*Cryptomeria japonica*	$[\alpha]_D$ −6.3°; b.p.$_9$ 145–150°	[(*5*), p. 756]
Lactaroviolin	(CCLXXI)	$C_{15}H_{14}O$	*Lactarius deliciosus*	m.p. 53°	[(*4*), p. 253; (*5*), p. 780]
Lactucin	(CCXCVIII)	$C_{15}H_{16}O_5$	*Lactuca virosa*	$[\alpha]_D$ +49° (methanol); m.p. 213–217°	(*58*)
Lanceol	(CCIV)	$C_{15}H_{24}O$	*Santalum lanceolatum* R. Br.	b.p.$_{17}$ 175–176°	[(*4*), p. 200]

Laserol	(CCCIV)	$C_{15}H_{26}O_5$	*Laserpitium latifolium* L.	m.p. 190°	(*59*)
Ledol	(CCLXXXI)	$C_{15}H_{26}O$	*Ledum palustre* L.; *Cistus labdanum Aristolochia indica; Phebalium squameus*	$[\alpha]_D^{20}$ +28° ($CHCl_3$); m.p. 104–105°	(*51*, *60*)
Linderene	(CCLXXVIII)	$C_{15}H_{18}O_2$	*Lindera strychnifolia*	m.p. 145°	[(*1*), Vol. V, p. 585; (*5*), p. 776
Longifolene	(CCLII)	$C_{15}H_{24}$	*Pinus longifolia* Roxb.; *P. mercusii; P. torreyana; P. maritima; P. densiflora*	$[\alpha]_D$ +42.7°; b.p.$_{36}$ 150–151°	[(*1*), Vol. V, p. 539; (*4*), p. 310; (*5*), p. 779; (*138*)]
Maaliol	(CCXLI	$C_{15}H_{26}O$	*Canarium samoense* Engl.	m.p. 103–104°	(*61*)
Matricarin	(CCXCVII)	$C_{17}H_{20}O_5$	*Matricaria chamomilla* L.	$[\alpha]_D^{20}$ +23°; m.p. 193–195°	(*62*)
Matricin	(CCLXXXVII)	$C_{17}H_{22}O_5$	*Matricaria chamomilla* L.	m.p. 158–160°	[(*4*), p. 267]
Metrosiderene	(CCXVII)	$C_{15}H_{24}$	*Dacrydium biforme*	$[\alpha]_D^{20}$ −2° (−8°); b.p.$_4$ 100°	[(*5*), p. 756]
Myoporone	(CXCVII)	$C_{15}H_{22}O_3$	*Myoporum bontioides* A. Gray	$[\alpha]_D$ 0°; b.p.$_{0.01}$ 117–119°	(*63*)
Nerolidol	(CXCII)	$C_{15}H_{26}O$	*Citrus aurantium* L. (Zingiberaceae, Leguminosae, Rutaceae)	$[\alpha]_D$ +15.5°; b.p.$_6$ 128–129°	[(*4*), p. 186]
Ngaione (*trans*)	(CXCV)	$C_{15}H_{22}O_3$	*Solanum tuberosum* L.	$[\alpha]_D$ −26.2°; b.p.$_{27}$ 182–183°	[(*4*), p. 188]
Nootkatin	(CCCXIX)	$C_{15}H_{20}O_2$	*Chamaecyparis nootkaensis; Cupressus macrocarpa; Juniperus chinensis*	m.p. 95–96°	[(*5*), p. 226]
Nortricycloekasantalal	(CCCXXVIII)	$C_{11}H_{16}O$	*Santalum album* L.	$[\alpha]_D$ −38.8°; b.p. 222–224°	[(*2*), Vol. II, p. 350]
Parthenin	(CCXCVI	$C_{15}H_{18}O_4$	*Parthenium hysterophorus* L.	$[\alpha]_D^{25}$ +7.02°; m.p. 163–166°	(*64*)
Partheniol	(CCLXXIV)	$C_{15}H_{24}O$	*Parthenium argentatum* Gray	m.p. 127–128°	[(*4*), p. 254]
Parthenolide	(CCLXVI)	$C_{15}H_{20}O_3$	*Chrysanthemum parthenium* (L.) Bernh.	$[\alpha]_D^{20}$ +81.4°; m.p. 115–116°	(*65*)
Patchouli alcohol	(CCLXXV)	$C_{15}H_{26}O$	*Pogostemon patchouly* var. *suavis*	$[\alpha]_D^{20}$ −124.5° (alcohol); m.p. 56°	[(*1*), Vol. III, p. 175; (*4*), p. 248]

TABLE XIV (*Continued*)

Compound	Number	Formula	Source	Physical Constants	References
Perezone	(CCX)	$C_{15}H_{20}O_3$	*Trixis pipitzahuac* Schaffner	$[\alpha]_D$ −17°; m.p. 102–104°	[(*4*), p. 202]
Petasin	(CCCV)	—	*Petasites hybridus* (L.) Fl. Wett.	—	(*66*)
Picrotoxinin	(CCCXX)	$C_{15}H_{16}O_6$	*Cocculus indicus*	m.p. 201°	(*67*)
Plumieride	(CCCXXXI)	$C_{21}H_{26}O_{12}$	Glucoside from *Plumiera* spp.	m.p. 224–225°	(*68*)
Pyrethrosin	(CCLXVII)	$C_{17}H_{22}O_5$	*Chrysanthemum cinerariaefolium*	$[\alpha]_D$ −31°; m.p. 360°	(*69*)
α-Santalene	(CCXLVIII)	$C_{15}H_{24}$	*Santalum album* L.	$[\alpha]_D$ −15°; b.p.$_9$ 118–120°	[(*4*), p. 238; (*5*), p. p. 766; (*139*)]
β-Santalene	(CCIL)	$C_{15}H_{24}$	*Santalum album* L.	$[\alpha]_D$ to −45°; b.p.$_9$ 125–127°	[(*5*), p. 766; (*139*)]
α-Santalol	(CCL)	$C_{15}H_{24}O$	*Santalum album* L.; *S. cygnorum*	$[\alpha]_D$ +1.3°; b.p.$_{10}$ 159–160°	[(*5*), p. 767; (*139*)]
β-Santalol	(CCLI)	$C_{15}H_{24}O$	*Santalum album* L.; *S. cygnorum*	$[\alpha]_D$ −57°; b.p.$_{10}$ 168–169°	[(*4*), p. 170; (*5*), p. 767; (*139*)]
α-Santonin	(CCXLV)	$C_{15}H_{18}O_3$	*Artemisia* spp.; *A. cina* B.; *A. maritima* L.; *A. gallica* Willd.; *A. brevifolia*	$[\alpha]_D^{18}$ −173° (alcohol); m.p. 170°	[(*4*), p. 221; (*5*), p. 763]
β-Santonin	(CCXLVI)	$C_{15}H_{18}O_3$	*Artemisia* spp.; *A. monogyna; A. salina; A. finita*	$[\alpha]_D^{19}$ −137.2° ($CHCl_3$); m.p. 216–218°	[(*4*), p. 221; (*5*), p. 764]
ψ-Santonin	(CCXLVII)	$C_{15}H_{20}O_4$	*Artemisia* spp.	$[\alpha]_D^{20}$ −169° ($CHCl_3$); m.p. 183–184°	[(*4*), p. 228; (*5*), p. 764]
β-Selinene	(CCXXX)	$C_{15}H_{24}$	*Apium graveolens* L.	$[\alpha]_D$ +38°; b.p.$_6$ 121°	[(*4*), p. 206]
Sesquibenihene	(CCXVI)	$C_{15}H_{24}$	*Chamaecyparis formosensis* Matsum	b.p.$_{10}$ 130–131°	[(*4*), p. 204; (*5*), p. 757]
Sesquibenihiol (Costol)	(CCXXXV)	$C_{15}H_{24}O$	*Saussurea lappa* Clarke	$[\alpha]_D$ +13°; b.p.$_{11}$ 169–171°	[(*4*), p. 238; (*70*)]

Sesquiterpene from vetiver	(CCXIV)	$C_{15}H_{24}$	South Indian vetiver oil	$[\alpha]_D$ +68.2°; b.p.$_2$ 94°	(*71*)
Temisin	(CCCXII)	$C_{15}H_{22}O_3$	*Artemisia maritima* L.	$[\alpha]_D^{20}$ +70° ($CHCl_3$); m.p. 228°	[(*5*), p. 761]
Tenulin	(CCXCIV)	$C_{17}H_{22}O_5$	*Helenium* spp.; *H. tenuifolium; H. elegans; H. badium; H. montanum*	$[\alpha]_D^{20}$ −21.6° (alcohol); m.p. 193–195°	[(*4*), p. 266]
Todomatsu acid	(CCIX)	$C_{15}H_{24}O_3$	*Abies sachalinensis*	$[\alpha]_D^{32}$ +85.8°; m.p. 58–58.5°	[(*5*), p. 397]
Torreyol	(CCXXIV)	$C_{15}H_{26}O$	*Torreya nucifera* Sieb. et. Zucc.	$[\alpha]_D$ +107.1°; m.p. 140°	[(*5*), p. 756]
Trichothecin	(CCCXXX)	$C_{18}H_{28}O_6$	*Trichothecium roseum* Link.	—	[(*5*), p. 298; (*72*)]
Tricyclovetivene	(CCCIII)	$C_{15}H_{24}$	*Vetivera zizanioides* Stapf.	$[\alpha]_D^{20}$ +13.1°; b.p.$_{0.6}$ 81–82°	[(*5*), p. 771]
Turmerone	(CCVII)	$C_{15}H_{22}O$	*Curcuma longa* L.; *Nectandra elaiphora*	b.p.$_1$ 114–117°	[(*4*), p. 202; (*5*), p. 752]
ar-Turmerone	(CCVIII)	$C_{15}H_{20}O$	*Curcuma longa* L.	$[\alpha]_D$ +83.8°; b.p.$_{10}$ 164°	[(*4*), p. 202; (*5*), p. 196]
β-Vetivone	(CCCII)	$C_{15}H_{22}O$	*Vetiveria zizanioides* Stapf.	$[\alpha]_D^{20}$ −38.9° (alcohol); m.p. 44–44.5°	[(*4*), p. 260]
Viridiflorol	(CCLXXXIII)	$C_{15}H_{26}O$	*Himantandra baccata* Bail.; *Juniperus oxycedrus*	m.p. 76°	(*48, 71*)
Xanthinin	(CCCXVIII)	$C_{17}H_{22}O_5$	*Xanthium pennsylvanicum*	m.p. 121–122°	(*73*)
Zerumbone	(CCLXII)	$C_{15}H_{22}O$	*Zingiber zerumbet*	m.p. 67–69°	(*74*)
Zierone	(CCCXVI)	$C_{15}H_{22}O$	*Zieria macrophylla* Bonpl.	$[\alpha]_D$ −141.2°; b.p.$_{18}$ 147–149°	[(*4*), p. 262]
(−)-Zingiberene	(CC)	$C_{15}H_{24}$	*Zingiber officinale* Rosc.; *Curcuma zedoaria* Rose; *C. longa* L.; *Thymus serphyllum* L.	$[\alpha]_D$ −69° to −73° b.p.$_{17}$ 137–139°	[(*4*), p. 194]

TABLE XV

Diterpene Compounds: Occurrence, Formulas, and Physical Constants

Compound	Number	Formula	Source	Physical constants	References
Abietic acid	(CCCLVIII)	$C_{20}H_{30}O_2$	*Pinus* spp.	$[\alpha]_D$ −106° (alcohol); m.p. 172–175°	[(*1*), Vol. III, p. 374; (*4*), Vol. III, p. 2]
Agathenedicarboxylic acid	(CCCXXXVIII)	$C_{20}H_{30}O_4$	*Dammara australis; D. orientalis* (kauri copal)	$[\alpha]_D$ +52° (alcohol); m.p. 204°	[(*1*), Vol. III, p. 459]
Ajaconine	(CCCXCVII)	$C_{22}H_{33}O_3N$	*Delphinium ajacis*	$[\alpha]_D^{18}$ −119 ($CHCl_3$); m.p. 172°	(*75*, *108*)
Andrographolide	(CCCXLVIII)	$C_{20}H_{30}O_5$	*Andrographis paniculata* Nees.	m.p. 228°	(*76*, *77*)
Aritasone	(CCCLXXXIX)	$C_{20}H_{28}O_2$	*Chenopodium ambrosioides*	$[\alpha]_D$ −118.8°; m.p. 105–106°	[(*5*), p. 221]
Artemisene	(CCCXXXVII)	$C_{20}H_{32}$	Wormwood oil	$[\alpha]_D$ −63°	(*78*)
Atisine	(CCCXCVI)	$C_{22}H_{33}O_2N$	*Aconitum heterophyllum* Wall.	$[\alpha]_D$ +28°; m.p. 57–60°	(*79*, *80*, *108*)
Cafestol	(CCCLXXXIV)	$C_{20}H_{28}O_3$	Coffee oil	$[\alpha]_D$ −103 to −107° ($CHCl_3$); m.p. 157–162°	[(*4*), Vol. III, p. 46; (*5*), p. 795; (*81*)]
α-Camphorene	(CCCXC)	$C_{20}H_{32}$	Camphor oil; *Cymbopogon citratus* (Lemongrass oil); *Dacrydium biforme*	b.p.$_{12}$ 190–192°	[(*2*), Vol. II, p. 126; (*5*), p. 781]
Cassaic acid	(CCCLXXXVI)	$C_{20}H_{30}O_4$	*Erythrophleum guinaense*	$[\alpha]_D$ −126°; m.p. 203°	(*82*, *83*, *84*)
Cativic acid	(CCCXXXIX)	$C_{20}H_{34}O_4$	*Prioria copaifera* Griseb. (cativo tree)	$[\alpha]_D$ −6.5° (alcohol); m.p. 80–82°	[(*4*), Vol. III, p. 20]
Columbin	(CCCXLVII)	$C_{20}H_{22}O_6$	*Iatrorrhiza palmata* Miers. (Colombo root)	$[\alpha]_D$ 0°; m.p. 182°	[(*4*), Vol. III, p. 54; (*85*)]

Cryptotanshinone	(CCCLXXVII)	$C_{19}H_{20}O_3$	*Salvia miltiorrhiza* Bge	$[\alpha]_D^{21}$ −91.4° (CHCl$_3$); m.p. 91°	(*107*)
Cuauchichicine	(CCCXCIII)	$C_{22}H_{33}O_2N$	*Garrya laurifolia* (Mexico)	$[\alpha]_D$ −71.4°; m.p. 152–155°	(*86*)
Dehydroabietic acid	(CCCLXI)	$C_{20}H_{28}O_2$	Wood rosin	$[\alpha]_D$ +64° (alcohol); m.p. 171–172°	[(*1*), Vol. III, p. 413]
Delpheline	(CCCIC)	$C_{25}H_{39}O_6N$	*Delphinium* spp. (Seed)	$[\alpha]_D$ −26° (alcohol); m.p. 216–219°	(*87*, *108*)
Dextropimaric acid	(CCCL)	$C_{20}H_{30}O_2$	*Pinus* spp.	$[\alpha]_D^{20}$ +75° (alcohol); m.p. 217–219°	[(*4*), Vol. III, p. 16]
Dextropimarinal	—	$C_{20}H_{30}O$	*Pinus* spp.; *P. sylvestris* L.; *P. palustris; P. elliotti*	m.p. 52°	[(*1*), Vol. V, p. 611; (5), p. 787]
Dihydroabietic acids	(CCCLXII)	$C_{20}H_{32}O_2$	Rosin	Mixture	[(*1*), Vol. III, p. 407]
Elliotinol	(CCCLXXI)	$C_{20}H_{32}O$	*Pinus elliotti* (gum rosin)	$[\alpha]_D$ +10.2°	(*88*)
Eperuic acid	(CCCXL)	$C_{20}H_{34}O_2$	*Eperua* spp. (wallaba tree)	b.p.$_{0.7}$ 190°	[(*4*), Vol. III, p. 20; (*95*)]
Ferruginol	(CCCLXIV)	$C_{20}H_{30}O_2$	Miro tree, *Dacrydium cupressinum; Juniperus communis; Podocarpus ferruginea; P. totara*	$[\alpha]_D^{16}$ +40.6° (alcohol); m.p. 57–59°	[(*4*), Vol. III, p. 22]
Fichtelite	(CCCLXXII)	$C_{19}H_{34}$	In old pine stumps	m.p. 46.5°	[(*4*), Vol. III, p. 36]
Garryfoline	(CCCXCV)	$C_{22}H_{33}NO_2$	*Garrya laurofolia*	$[\alpha]_D$ −60°; m.p. 130–133°	(*86*, *108*)
Garryine	(CCCXCI)	$C_{22}H_{32}O_2N$	*Garrya veatchii* Kellogg	m.p. 96°	(*108*)
Gibberellic acid	(CCCLXXXIII)	$C_{19}H_{22}O_6$	*Gibberella fujikuroi* (Saw) Wr.	$[\alpha]_D$ +93°; m.p. 233–235°	(*89*, *90*)
Grayanol	(CCCLXXVIII)	$C_{20}H_{32}O_5$	*Leucothoë grayana*	$[\alpha]_D$ −42°; m.p. 198°	[(*5*), p. 792]
Hetisine	(CCCXCVIII)	$C_{20}H_{27}O_3N$	*Aconitum heterophyllum*	$[\alpha]_D$ +13.7 (alcohol); m.p. 253–254°	(*91*, *108*)

TABLE XV (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
Hinokiol	(CCCLXVI)	$C_{20}H_{30}O_2$	*Chamaecyparis obtusa* Sieb. et Zucc.	$[\alpha]_D^{20}$ +74.4°; m.p. 234–235°	[(*4*), Vol. III, p. 24; (*137*)]
Hinokione	(CCCLXVII)	$C_{20}H_{28}O_2$	Hinoki oil	$[\alpha]_D^{20}$ +103.4°; m.p. 188–189°	[(*5*), p. 786; (*137*)]
Isodextropimaric acid	(CCCLII)	$C_{20}H_{30}O_2$	*Pinus* spp.; rosin; *Podocarpus ferruginea; Dacrydium biforme; D. kirkii*	$[\alpha]_D$ 0°; m.p. 162–164°	[(*4*), Vol. III, p. 16]
7-Isodextropimaric acid	(CCCLIV)	$C_{20}H_{30}O_2$	*Juniperus rigida*	$[\alpha]_D$ −18.9°; m.p. 162.5–165°	(*92*)
Isodextropimarinal	(CCCLIII)	$C_{20}H_{30}O$	*Pinus* spp.; rosin; *P. palustris; P. elliotti*	m.p. 50–52°	[(*5*), p. 787]
Kahweol	(CCCLXXXV)	$C_{20}H_{24}O_3$	Coffee oil	—	[(*5*), p. 795; (*93*)]
Kaurene	(CCCLXXX)	$C_{20}H_{32}$	*Agathis australis* Salisb.	—	(*94*)
9-Ketoferruginol (sugiol)	(CCCLXV)	$C_{20}H_{28}O_2$	*Cryptomeria japonica* Don.; *Dacrydium cupressinum; Juniperus communis*	$[\alpha]_D^{20}$ +20–22° (alcohol); m.p. 283–290°	[(*5*), p. 786]
Ketomanoyl oxide	(CCCXLIV)	$C_{20}H_{32}O_2$	*Dacrydium colensoi*	$[\alpha]_D^{13}$ +40.4° (alcohol); m.p. 76°	[(*4*), Vol. III, p. 32]
Labdanolic acid	(CCCXLI)	$C_{20}H_{30}O_2$	Gum labdanum	$[\alpha]_D$ +13.5°; m.p. 114–115°	[(*4*), Vol. III, p. 20; (*95*)]
Levopimaric acid	(CCCLVI)	$C_{20}H_{30}O_2$	*Pinus* spp.; oleoresins	$[\alpha]_D$ −276° (alcohol); m.p. 150–152°	[(*4*), Vol. III, p. 12]
Lycoctonine	(CD)	$C_{20}H_{33}NO_5$	*Delphinium ajacis*	m.p. 110–115°	(*96*, *108*)
Manool	(CCCXLII)	$C_{20}H_{34}O$	*Dacrydium* spp.; *D. biforme; D. kirkii; D. bidwillii*	$[\alpha]_D^{19}$ +30.4° (alcohol); m.p. 53°	[(*1*), Vol. III, p. 350]

Manoyl oxide	(CCCXLIII)	$C_{20}H_{34}O$	*Dacrydium colensoi* (silver pine)	$[\alpha]_D^{13}$ +19.6° (alcohol); m.p. 29°	[(*4*), Vol. III, p. 40]
Marrubiin	(CCCXLVI)	$C_{20}H_{28}O_4$	*Marrubium vulgare; Ballota foetida*	$[\alpha]_D^{20}$ +45° (alcohol); m.p. 158–160°	[(*4*), Vol. III, p. 38; (*5*), p. 791]
Mirene	(CCCLXXXI)	$C_{20}H_{32}$	*Podocarpus ferruginea*	$[\alpha]_D$ +43.8°; m.p. 59–60°	(*94*, *97*)
Nappeline	(CCCXCIV)	$C_{22}H_{33}NO_3$	*Aconitum napellum*	m.p. 85–88°	(*108*)
Neoabietic acid	(CCCLVII)	$C_{20}H_{30}O_2$	*Pinus* spp.; rosin	$[\alpha]_D$ +159°; m.p. 167–169°	[(*4*), Vol. III, p. 14]
Nimbiol	(CCCLXIX)	$C_{18}H_{24}O_2$	*Melia azadirachta*	$[\alpha]_D$ +32.3° ($CHCl_3$); m.p. 244°	(*98*)
Palustric acid	(CCCLIX)	$C_{20}H_{30}O_2$	*Pinus* spp.; rosin	$[\alpha]_D$ +71.6°; m.p. 162–167°	(*99*)
Phyllocladene	(CCCLXXIX)	$C_{20}H_{32}$	*Phyllocladus* spp.; *Dacrydium biforme; D. colensoi; Araucaria excelsa*	$[\alpha]_D$ +15.8°; m.p. 98°	[(*4*), Vol. III, p. 36; (*94*)]
Phytol	(CCCXXXV)	$C_{20}H_{40}O$	Chlorophyll; vitamin E; vitamin K_1	—	[(*5*), p. 54]
Podocarpic acid	(CCCLXX)	$C_{17}H_{22}O_3$	*Podocarpus* spp.; *P. cupressina; P. dacrydioides*	$[\alpha]_D$ +136° (alcohol) m.p. 187–193°	[(*4*), Vol. III, p. 20; (*5*), p. 790]
Retene	(CCCLXXIII)	$C_{18}H_{18}$	In old pine stumps	m.p. 98°	(*100*)
Rimuene (totarene)	(CCCIL)	$C_{20}H_{32}$	*Dacrydium cupressinum; Podocarpus totara*	$[\alpha]_D$ +44.7°; m.p. 55°	[(*5*), p. 784; (*94*)]
Rosenonolactone	(CCCLV)	$C_{20}H_{28}O_3$	Mycelium from *Trichothecium roseum* Link.	m.p. 208°, $[\alpha]_D$ −118°	(*101*, *102*)
Sandaracopimaric acid	(CCCLI)	$C_{20}H_{30}O_2$	Sandarac resin	m.p. 172°	[(*4*), Vol. III, p. 20; (*103*, *141*)]
Sclareol	(CCCXLV)	$C_{20}H_{36}O_2$	*Salvia sclarea* L.	$[\alpha]_D^{20}$ −6.2° (alcohol); m.p. 104–106°	[(*4*), Vol. III, p. 32; (*104*)]

TABLE XV (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
Steviol	(CCCLXXXII)	$C_{20}H_{30}O_3$	*Stevia rebaudiana* Bertoni	$[\alpha]_D$ −94° (alcohol); m.p. 212–217°	[(*4*), Vol. III, p. 52; (*105*)]
Tanshinone I	(CCCLXXV)	$C_{18}H_{12}O_3$	*Salvia miltiorrhiza* Bge.	m.p. 232–234°	(*106*, *107*)
Tanshinone II	(CCCLXXVI)	$C_{19}H_{18}O_3$	*Salvia miltiorrhiza* Bge.	m.p. 216°	(*107*)
Teloabietic acid (=isoabietic acid)	(CCCLX)	$C_{20}H_{30}O_2$	*Pinus* spp.; oleoresins	$[\alpha]_D$ +60°; m.p. 151–153°	(*12*)
Tetrahydroabietic acid	(CCCLXIII)	$C_{20}H_{34}O_2$	*Pinus* spp.; rosin	Mixture	[(*1*), Vol. III, pp. 384, 418, 426]
Totarol	(CCCLXVIII)	$C_{20}H_{30}O$	*Podocarpus* spp.; *P. totara; P. ferruginea; Dacrydium cupressinum*	$[\alpha]_D$ +41.3° (alcohol); m.p. 132°	[(*1*), Vol. III, p. 358; (*4*), Vol. III, p. 24]
Veatchine	(CCCXCII),	$C_{22}H_{32}O_2N$	*Garrya veatchii* Kellogg	m.p. 122–123°	(*108*)
Vinhaticoic acid	(CCCLXXXVII)	$C_{20}H_{28}O_3$	*Plathymenia reticulata* Benth.	$[\alpha]_D$ +73°; m.p. 153–154°	[(*4*), Vol. III, p. 24]
Vitamin A	(CCCXXXVI)	$C_{20}H_{30}O$	Fish liver oils	m.p. 7.5–8°	
Vuocapenic acid	(CCCLXXXVII)	$C_{21}H_{30}O_3$	*Vouacapoua* spp.; *V. americana* Aubl.; *V. macropetala*	$[\alpha]_D$ +101°; m.p. 103–104°;	[(*4*), Vol. III, p. 30]
Xanthoperol	(CCCLXXIV)	$C_{20}H_{26}O_3$	*Juniperus communis* L.	$[\alpha]_D$ +132.5° (±2.5°); m.p. 255–270°	(*109*)

TABLE XVI

TRITERPENE COMPOUNDS: OCCURRENCE, FORMULAS, AND PHYSICAL CONSTANTS

Compound	Number	Formula	Source	Physical constants	References
Agnosterol	(CDXV)	$C_{30}H_{48}O$	Wool fat	$[\alpha]_D$ +65.9°; m.p. 164.5–165.5°	[(*4*), Vol. III, p. 76]
Albigenic acid	(CDLXXXVII)	$C_{30}H_{48}O_4$	*Albizzia lebbeck* Benth. (beans)	—	(*110*)
Alnusenone (=Glutinone	(DVI)	$C_{30}H_{48}O$	Bark of black alder	$[\alpha]_D$ +31° ($CHCl_3$); m.p. 247°	[(*4*), Vol. III, p. 200; (*5*), p. 832]
Ambrein	(CDXI)	$C_{30}H_{52}O$	Ambergris	$[\alpha]_D$ +20.5°; m.p. 83°	[(*4*), Vol. III, p. 70]
α-Amyrin	(XDIV)	$C_{30}H_{50}O$	Manila elemi resin; latex from milk tree (*Brosium galactodendrum*)	$[\alpha]_D$ +83° ($CHCl_3$) m.p. 186°	[(*4*), Vol. III, pp. 156, 166; (*5*), p. 821]
β-Amyrin	(CDLI)	$C_{30}H_{50}O$	Elemi resin; shea nut fat; balata gum; gutta-percha; holly birdlime	$[\alpha]_D^{20}$ +88° ($CHCl_3$); m.p. 197–200°	[(*4*), Vol. III, p. 153; (*5*), p. 798]
Arjunolic acid	(CDLII)	$C_{30}H_{48}O_5$	*Terminalia arjuna*	$[\alpha]_D^{19}$ +63.5° (alcohol); m.p. 337–340°	[(*4*), Vol. III, p. 154; (*5*), p. 815]
Asiatic acid	(XDV)	$C_{30}H_{48}O_3$	*Hydrocotyle asiatica*	$[\alpha]_D$ +51° (alcohol); m.p. 300–305°	[(*4*), Vol. III, p. 168; (*5*), p. 825]
A_1-Barrigenol	(CDLXXV)	$C_{30}H_{50}O_5$	*Barringtonia asiatica; Pittosporum undulatum*	$[\alpha]_D$ +4°; m.p. 300–302°	[(*5*), p. 805]
Barringtogenol	(CDLIII)	$C_{30}H_{50}O_4$	*Barringtonia racemosa*	$[\alpha]_D$ +18° (pyridine); m.p. 290–291°	[(*4*), Vol. III, p. 154; (*5*), p. 804]
Barringtogenolic acid	(CDLV)	$C_{30}H_{46}O_6$	*Barringtonia racemosa* Roxb.	$[\alpha]_D$ +72° (methanol); m.p. 332–334°	[(*5*), p. 820]

TABLE XVI (*Continued*)

Compound	Number	Formula	Source	Physical constants	References
Bassic acid	(CDLXXXII)	$C_{30}H_{46}O_5$	*Mimusops* spp.; *M. heckelii; M. hexandra* Roxb.; *Bassia* spp.; *B. latifolia; B. butyracea; B. parkii; Dummoria heckelii* Pierre	$[\alpha]_D$ +82.4° (pyridine); m.p. 316°	[(*5*), p. 815; (*111*)]
Bauerenol	(DIX)	$C_{30}H_{50}O$	—	—	(*112*)
Betulafolientriol	(CDXLII)	$C_{30}H_{52}O_3$	Birch (leaves)	m.p. 112–121°	(*113*)
Betulin	(CDXLIV)	$C_{30}H_{50}O_2$	*Betula alba* (bark); *B. platyphylla; Zizyphus vulgaris; Corylus avellana; Trochodendron aralioides*	$[\alpha]_D^{15}$ +20° (pyridine); m.p. 251–252°	[(*5*), p. 829; (*4*), Vol. III, p. 172]
Betulinic acid	(CDXLV)	$C_{30}H_{48}O_3$	*Platanus orientalis* L.; *Gratiola officinalis* L.; *Cornus florida* L.; *Lemaireocereus hystrix; Punica granatum*	$[\alpha]_D$ +8° (pyridine); m.p. 316–320°	[(*5*), p. 829; (*4*), Vol. III, p. 172]
α-Boswellic acid	(CDLXXVIII)	$C_{30}H_{48}O_3$	Olibanum from *Boswellia carteri* and *B. frereana*	$[\alpha]_D^{19}$ +115° ($CHCl_3$); m.p. 289°	[(*4*), Vol. III, p. 154; (*5*), p. 808]
β-Boswellic acid	(DI)	$C_{30}H_{48}O_3$	Olibanum	$[\alpha]_D$ +119° ($CHCl_3$); m.p. 238–240°	[(*5*), p. 825; (*4*), Vol. III, p. 168]
Brein	(XDVIII)	$C_{30}H_{50}O_2$	Manila elemi resin	$[\alpha]_D$ +66° (alcohol); m.p. 221–223°	[(*4*), Vol. III, p. 168; (*5*), p. 823]
Butyrospermol	(CDXXXIII)	$C_{30}H_{50}O$	Shea nut fat from *Butyrospermum parkii*	$[\alpha]_D$ −12° ($CHCl_3$); m.p. 111–113°	[(*4*), Vol. III, p. 110; (*5*), p. 836; (*114, 115, 144*)]
Cerin	(DVII)	$C_{30}H_{50}O_2$	Cork	$[\alpha]_D$ −44.5° ($CHCl_3$); m.p. 247–251°	[(*4*), Vol. III, p. 212; (*5*), p. 833]
Chichipegenin	(CDLX)	$C_{30}H_{50}O_4$	*Myrtillocactus* spp	$[\alpha]_D$ +43° ($CHCl_3$); m.p. 321–323°	(*116*)

Citrostadienol	(DXIII)	$C_{30}H_{50}O$	Israeli grapefruit; orange peel oil	$[\alpha]_D$ +24° ($CHCl_3$); m.p. 162–164°	*(117)*
Cochalic acid	(CDLXXXI)	$C_{30}H_{48}O_4$	*Myrtillocactus cochal*	$[\alpha]_D$ +85° (dioxan); m.p. 303–304°	[(*5*), p. 810]
Crataegolic acid	(CDLXXXV)	$C_{30}H_{48}O_4$	*Crataegus oxycantha* L.; *Psidium guajava*	CH_3—ester: $[\alpha]_D^{20}$ +67.5 ± 3° ($CHCl_3$); m.p. 223–227°	[(*5*), p. 812; (*118*)]
Cycloartenol	(CDXXIX)	$C_{30}H_{50}O$	*Euphorbia* spp.; *E. handiensis; E. obtusifolia; E. balsamifera; E. aphyla*	$[\alpha]_D$ 48–54°; m.p. 111–115°	[(*4*), Vol. III, p. 92; (*5*), p. 836]
Cycloeucalenol	(DXII)	$C_{30}H_{50}O$	*Eucalyptus microcorys*	m.p. 140°	*(119)*
Cyclolaudenol	(CDXXXI)	$C_{31}H_{52}O$	Opium	$[\alpha]_D$ +46° ($CHCl_3$); m.p.125°	[(*4*), Vol. III, p. 92; (*5*), p. 837]
Cycloorystenol	(CDXXX)	$C_{30}H_{50}O$	Rice bran oil	$[\alpha]_D$ +49°; m.p. 112–113°	*(120)*
Dammadienol	(CDXL)	$C_{30}H_{50}O$	Dammar resin	$[\alpha]_D$ +47°; m.p. 136–138°	[(*4*), Vol. III, p. 114; (*121*)]
Dammarenediol I and II	(CDXLI)	$C_{30}H_{52}O_2$	Dammar resin	I: $[\alpha]_D$ +27°; m.p. 142–144° II: $[\alpha]_D$ +33°; m.p. 131–133°	*(121)*
Dammarenolic acid	(DXI)	$C_{30}H_{52}O_3$	Dammar resin	$[\alpha]_D$ +45°; m.p. 138–139°	*(122, 123)*
Dehydroeburicoic acid	(CDXXVII)	$C_{31}H_{48}O_3$	*Lentinus dactyloides; Poria cocos* Wolf; *Polyporus hispidus*	$[\alpha]_D^{23}$ +40° ($CHCl_3$)	[(*4*), Vol. III, p. 95; (*5*), p. 841]
Dehydrotumulosic acid	(CDXXIV)	$C_{31}H_{48}O_4$	*Polyporus* spp.; *P. tumulosus; P. australiensis; Poria cocos* Wolf	—	[(*5*), p. 842]
Dumortierigenin	(CDLXIII)	$C_{30}H_{46}O_4$	*Lemaireocereus damortieri*	$[\alpha]_D^{17}$ −18.6°; m.p. 292–295°	[(*4*), Vol. III, p. 156; (*5*), p. 812]
Eburicoic acid	(CDXXVI)	$C_{31}H_{50}O_3$	*Fomes officinalis; Lentinus dactyloides; Poria cocos* Wolf; *Polyporus hispidus*	$[\alpha]_D^{30}$ +50° ($CHCl_3$); m.p. 292°	[(*4*), Vol. III, p. 94; (*5*), p. 840]

TABLE XVI (*Continued*)

Compound	Number	Formula	Source	Physical Constants	References
Echinocystic acid	(CDLXXX)	$C_{30}H_{48}O_4$	*Echinocystis fabacca*	$[\alpha]_D^{26}$ +27.7° (alcohol); m.p. 305–312°	[(*4*), Vol. III, p. 154; (*5*), p. 810]
α-Elemolic acid	(CDXXXVI)	$C_{30}H_{48}O_3$	Manila elemi resin from *Canarium commune* L.; *C. schweinfurthii*	$[\alpha]_D$ −25.6°; m.p. 224–226°	[(*4*), Vol. III, p. 102; (*5*), p. 838]
β-Elemonic acid	(CDXXXVII)	$C_{30}H_{46}O_3$	Manila elemi resin	$[\alpha]_D^{20}$ +46° ($CHCl_3$); m.p. 224–225°	[(*4*), Vol. III, p. 102; (*5*), p. 838]
Entagenic acid	(CDLVIII)	$C_{30}H_{48}O_5$	*Entada phaseoloides* Merrill	m.p. 310–315°	(*124*)
Erythrodiol	(CDLXIX)	$C_{30}H_{50}O_2$	*Erythroxylum novogranatense; Lemaireocereus longispinus; L. hystrix; L. griseus*	$[\alpha]_D$ +76–80° ($CHCl_3$); m.p. 235–237°	[(*4*), Vol. III, p. 154; (*5*), p. 801]
Euphol	(CDXXXII)	$C_{30}H_{50}O$	*Euphorbia* spp.; *E.* resin; *E. resinifera; E. triangularis; E. tirucalli; E. handiensis*	$[\alpha]_D$ +32° ($CHCl_3$); m.p.115–117°	[(*4*), Vol. III, p. 102; (*5*), p. 835]
Euphorbol	(CDXXXV)	$C_{31}H_{52}O$	*Euphorbia* spp.; *E. triangularis; E. ingens*	m.p. 126°	[(*4*), Vol. III, p. 102; (*5*), p. 836]
Friedelin	(DVIII)	$C_{30}H_{50}O$	Cork; *Balanops australiana; Salix japonica; Rhododendron westlandii*	$[\alpha]_D^{14}$ −22° to −27.8°; m.p. 257–265°	[(*4*), Vol. III, p. 202; (*5*), p. 832]
Germanicol	(XDIII)	$C_{30}H_{50}O$	*Lactuca virosa; Euphorbia balsamifera*	$[\alpha]_D$ +5.8° ($CHCl_3$); m.p. 177–178°	[(*4*), Vol. III, p. 176; (*5*), p. 798]
Glabrinic acid	(CDLXI)	$C_{30}H_{46}O_5$	*Glycyrrhiza glabra* L.	$[\alpha]_D$ −26° (pyridine); m.p. 329–333°	(*125*)
Glycyrrhetinic acid	(CDLXII)	$C_{30}H_{46}O_4$	Liquorice root; *Glycyrrhiza glabra* L.; *G. echinata; Periandra dulcis; P. mediterranea*	$[\alpha]_D$ +161° ($CHCl_3$); m.p. 297–298°	[(*4*), Vol. III, p. 154; (*5*), p. 818]
Gossypol	(DXV)	$C_{30}H_{30}O_8$	*Gossypium* spp.	m.p. 184° (and 199° and 214°)	[(*5*), p. 165]

Gratiogenin	(CDLXXVII)	$C_{30}H_{48}O_4$	*Gratiola officinalis*	$[\alpha]_D$ +168° (CHCl$_3$); m.p. 191–196°	[(*5*), p. 805]
Gummosogenin	(CDLXXVI)	$C_{30}H_{48}O_3$	*Machaerocereus gummosus*	$[\alpha]_D^{25}$ +28° (CHCl$_3$); m.p. 251–252°	[(*4*), Vol. III, p. 156; (*5*), p. 805]
Gypsogenin	(CDLXXXVIII)	$C_{30}H_{46}O_4$	Fuller's herb; *Agrostemma githago* L.; *Saponaria officinalis; Spergularia marginata*	$[\alpha]_D$ +91° (alcohol); m.p. 274–276°	[(*4*), Vol. III, p. 154; (*5*), p. 816; (*5*), p. 817]
Hederagenin	(CDLXVII)	$C_{30}H_{48}O_4$	*Sapindus* spp.; *S. saponaria* L.; *S. mukorossi utilis; Hedera helix; Nigella sativa* L.; *Clematis paniculata*	$[\alpha]_D$ +81° (pyridine); m.p. 332–334°	[(*4*), Vol.III, p. 130; (*5*), p. 813]
Hydroxyhopanone	(CDIL)	$C_{30}H_{50}O_2$	Dammar resin	$[\alpha]_D$ +69° (CHCl$_3$); m.p. 255°	(*125*, *127*)
3β-Hydroxylanosta-8,24-dien-21-oic acid	(CDXXI)	$C_{30}H_{48}O_3$	*Trametes odorata* (Wulf) Fr.	$[\alpha]_D$ +43°; m.p. 253–258°	(*128*)
21-Hydroxylanosta-7,9(11),24-trien-3-one	(CDXX)	$C_{30}H_{46}O_2$	*Polyporus pinicola* Fr.	$[\alpha]_D$ +56°; m.p. 119–121°	(*129*)
Icterogenin A and B	(XDI)	$C_{35}H_{52}O_6$	*Lippia rehmanni*	A: m.p. 239°; $[\alpha]_D$ +63° B: m.p. 155–160°; $[\alpha]_D$ +63°	[(*4*), Vol. III, p. 152; (*5*), p. 820]
Isomasticadienonic acid	(CDXXXIX)	$C_{30}H_{46}O_3$	Gum mastic	$[\alpha]_D$ +34° (CHCl$_3$); m.p. 166–167°	[(*5*), p. 839]
Lanosta-7,9(11),24-triene-3β,21-diol	(CDXIX)	$C_{30}H_{48}O_2$	*Polyporus pinicola* Fr.	$[\alpha]_D$ +22°; m.p. 194–197°	(*129*)
Lanosterol	(CDXIV)	$C_{30}H_{50}O$	Wool fat; yeast; *Euphorbia balsamifera*	$[\alpha]_D^{20}$ 59–61° (CHCl$_3$); m.p. 138–140°	[(*4*), Vol. III, p. 76; (*5*), p. 834]
Lantadene B	(CDLXV)	$C_{35}H_{52}O_5$	*Lantana camera*	$[\alpha]_D$ +85° (methanol); m.p. 293°	[(*4*), Vol. III, p. 154; (*5*), p. 819]

TABLE XVI (*Continued*)

Compound	Number	Formula	Source	Physical Constants	References
Longispinogenin	(CDLXXI)	$C_{30}H_{50}O_3$	*Lemaireocereus longispinus; L. griseus; L. hystrix* L.; *L. quevedonis; Escontria chiotilla*	$[\alpha]_D^{25}$ 51–54° (CHCl$_3$); m.p. 247–249°	[(*4*), Vol. III, p. 156; (*5*), p. 802]
Lophenol	(DXIV)	$C_{28}H_{48}O$	*Lophocereus schottii*	$[\alpha]_D$ +5° (CHCl$_3$); m.p. 149–151°	(*130*)
Lupeol	(CDXLIII)	$C_{30}H_{50}O$	*Lupinus luteus; Achras sapota; Ficus* spp.; *Crataera* spp.	$[\alpha]_D$ +27° (CHCl$_3$); m.p. 212–215°	[(*5*), p. 828]
Machaeric acid	(CDLXXXIV)	$C_{30}H_{46}O_4$	*Machaerocereus gummosus*	$[\alpha]_D$ +20°; m.p. 309–312°	[(*4*), Vol. III, p. 156; (*5*), p. 811]
Machaerinic acid	(CDLXXXIII)	$C_{30}H_{48}O_4$	*Machaerocereus gummosus*	CH$_3$-ester; m.p. 232–234°	[(*4*), Vol. III, p. 156; (*5*), p. 811]
Maniladiol	(CDLXVIII)	$C_{30}H_{50}O_2$	Manila elemi resin; *Escontria chiotilla*	$[\alpha]_D$ +68°; m.p. 221°	[(*4*), Vol. III, p. 154; (*5*), p. 800]
Masticadienonic acid	(CDXXXVIII)	$C_{30}H_{46}O_3$	Gum mastic	$[\alpha]_D$ −76° (CHCl$_3$); m.p. 178°	[(*4*), Vol. III, p. 110; (*5*), p. 839]
Medicagenic acid	(CDLVI)	$C_{30}H_{46}O_6$	*Medicago sativa*	$[\alpha]_D$ +111° (alcohol); m.p. 349–350°	(*131*)
Melaleucinic acid	(CDXLVI)	$C_{30}H_{46}O_5$	*Melaleuca* spp.	m.p. 363–364°	[(*5*), p. 830]
Morolic acid	(XDII)	$C_{30}H_{48}O_3$	*Mora excelsa* Benth.	$[\alpha]_D$ +31° (CHCl$_3$); m.p. 273°	[(*4*), Vol. III, p. 148; (*5*), p. 809]
Myrtillogenic acid	(CDLVII)	$C_{30}H_{48}O_5$	*Myrtilluscactus* spp.	$[\alpha]_D$ +84° (CHCl$_3$); m.p. 288–293°	(*132*)
Nyctanthic acid	(DX)	$C_{30}H_{48}O_2$	*Nyctanthes arbortristis*	m.p. 222.5–223.5°	(*123*)
Obtusifoldienol	(CDXXV)	$C_{31}H_{52}O$	*Euphorbia obtusifolia* Poir.	$[\alpha]_D$ +72.1°; m.p. 125–130°	(*133*)
Oleanolic acid	(CDLIX)	$C_{30}H_{48}O_3$	Clove buds; sugar beets; of many plants; *Olea europaea* L.; *Viscum album; Salvia officinalis; Vaccinium myrtillis; Lemaireocereus* spp.	$[\alpha]_D$ +80° (methanol); m.p. 305–310°	[(*4*), Vol. III, p. 153; (*5*), p. 806]

α-Onocerin	(CDXII)	$C_{30}H_{50}O_2$	*Ononis spinosa*	$[\alpha]_D$ +18° ($CHCl_3$); m.p. 202–203°	[(*4*), Vol. III, p. 120; (*5*), p. 843]
Parkeol	(CDXVI)	$C_{30}H_{50}O$	*Butyrospermum parkii*	$[\alpha]_D$ +65°; m.p. 164°	(*134*)
Phyllantol	(DIII)	$C_{30}H_{50}O$	*Phyllantuus engleri* Pax.	$[\alpha]_D$ +43° ($CHCl_3$); m.p. 233–234°	[(*4*), Vol. III, p. 168; (*5*), p. 827]
Pinicolic acid	(CDXVII)	$C_{30}H_{46}O_3$	*Polyporus pinicola*	$[\alpha]_D$ +68° ($CHCl_3$); m.p. 197–202°	[(*4*), Vol. III, p. 96; (*5*), p. 842]
Polyporenic acid A	(CDXXVIII)	$C_{31}H_{50}O_4$	*Polyporus betulinus*	$[\alpha]_D$ +69° (pyridine); m.p. 194–199°	[(*4*), Vol. III, p. 94; (*5*), p. 840]
Polyporenic acid C	(CDXXII)	$C_{31}H_{46}O_4$	*Polyporus betulinus; P. benzoinus*	$[\alpha]_D$ +6° (pyridine); m.p. 273–276°	[(*4*), Vol. III, p. 96; (*5*), 839]
Primulagenin A	(CDLXX)	$C_{30}H_{50}O_3$	*Primula officinalis* (roots); *P. elatior*	$[\alpha]_D$ +52° ($CHCl_3$); m.p. 248–250°	[(*5*), p. 801]
Queretaroic acid	(CDLXXXVI)	$C_{30}H_{48}O_4$	*Lemaireocereus queretaroensis; L. beneckei*	m.p. 318–323°	[(*4*), Vol. III, p. 156; (*5*), p. 814]
Quillaic acid	(CDLXXXIX)	$C_{30}H_{46}O_5$	*Quillaia* bark	$[\alpha]_D$ +56.1° (pyridine); m.p. 292–294°	[(*5*), p. 817]
Quinovic acid	(DII)	$C_{30}H_{46}O_5$	Cinchona bark; *Potentilla tormentilla* (root); *Zygophyllum coccineum* L.; *Mitragyna* spp. (leaves)	$[\alpha]_D^{16}$ +87° (KOH); m.p. 298°	[(*4*), Vol. III, p. 168; (*5*), p. 826]
Rehmannic acid (=Lantadene A)	(CDLXIV)	$C_{35}H_{52}O_5$	*Lantana camara; Lippia rehmanni* (root)	$[\alpha]_D$ +84° ($CHCl_3$); m.p. 295–300°	[(*4*), Vol. III, p. 154; (*5*), p. 819]
Siaresinolic acid	(CDLXVI)	$C_{30}H_{48}O_4$	Siam gum benzoin	$[\alpha]_D$ +98.5° ($CHCl_3$); m.p. 279–280°	[(*4*), Vol. III, p. 142; (*5*), p. 811]
Soysapogenol A	(CDLXXII)	$C_{30}H_{50}O_4$	Soysapogenin; *Trifolium repens*	$[\alpha]_D$ +103° ($CHCl_3$); m.p. 321°	[(*5*), p. 802]

TABLE XVI (*Continued*)

Compound	Number	Formula	Source	Physical Constants	References
Soysapogenol B	(CDLXXIII)	$C_{30}H_{50}O_3$	Soysapogenin; *Trifolium repens*	$[\alpha]_D$ +92° ($CHCl_3$); m.p. 259–260°	[(*5*), p. 803]
Soysapogenol C	(CDLXXIV)	$C_{30}H_{48}O_2$	Soysapogenin; *Trifolium repens*	$[\alpha]_D$ +71° ($CHCl_3$); m.p. 239–240°	[(*5*), p. 803]
Soysapogenol D	(CDXIII)	$C_{30}H_{50}O_3$	Soya	$[\alpha]_D$ −61° ($CHCl_3$); m.p. 298°	[(*5*), p. 804]
Squalene	(CDX)	$C_{30}H_{50}$	Fish oils; plant oils; *Torula utilis*	b.p.$_{10}$ 262–264°	[(*4*), Vol. III, p. 68; (*5*), p. 23]
Stellatogenin	(CDXLVIII)	$C_{30}H_{48}O_4$	*Lemaireocereus stellatus; L. treleasi*	$[\alpha]_D^{22}$ +36° ($CHCl_3$); m.p. 317–319°	[(*4*), Vol. III, p. 178; (*5*), p. 831]
Sumaresinolic acid	(CDLXXIX)	$C_{30}H_{48}O_4$	Sumatra gum benzoin	$[\alpha]_D$ +54° ($CHCl_3$); m.p. 298°	[(*4*), Vol. III, p. 154; (*5*), p. 809]
Taraxasterol α and β	(XDVI)	$C_{30}H_{50}O$	*Taraxacum officinale; Anthemis nobilis; Souchus arvensis* L.; *Lactuca virosa* L.; *L. sativa* L.; *Cichorium endivia; Calotropis procera*	α: $[\alpha]_D$ +96°; m.p. 220–222° β: $[\alpha]_D$ +48°; m.p. 168–180°	[(*4*), Vol. III, p. 182; (*5*), p. 821]
ψ-Taraxasterol	(XDVII)	$C_{30}H_{50}O$	*Taraxacum officinale; T. kok-saghyz; Cynara scolymus*	$[\alpha]_D$ 45–50° ($CHCl_3$); m.p. 217–219°	[(*4*), Vol. III, p. 182; (*5*), p. 821]
Taraxerol	(DIV)	$C_{30}H_{50}O$	*Skimmia* spp.; *Alnus* spp.; *Tilia cordata* (bark); *Myrica rubra; Taraxacum kok-saghyz*	$[\alpha]_D$ +3° ($CHCl_3$); m.p. 279–282°	[(*4*), Vol. III, p. 198; (*5*), p. 799]
Taraxerone	(DV)	$C_{30}H_{48}O$	*Alnus glutinosa; A. viridis; A. indica* (bark)	$[\alpha]_D^{20}$ −11° ($CHCl_3$); m.p. 240–241°	[(*5*), p. 800]
Terminolic acid	(CDLIV)	$C_{30}H_{48}O_6$	*Terminalia ivorensis*	$[\alpha]_D$ +42° (alcohol); m.p. 347°	[(*4*), Vol. III, p. 154; (*5*), p. 815]

Thurberogenin	(CDXLVII)	$C_{30}H_{46}O_3$	*Lemaireocereus* spp.; *L. thurberi; L. stellatus*	$[\alpha]_D^{22}$ +11° (CHCl$_3$); m.p. 283–285°	[(*5*), p. 831]
Tirucallol	(CDXXXIV)	$C_{30}H_{50}O$	*Euphorbia tirucalli* (resin); *E. triangularis*	$[\alpha]_D$ +4.5° (benzene); m.p. 133–134.5°	[(*4*), Vol. III, p. 102; (*5*), p. 835]
Trametenolic acid B	(CDXVIII)	$C_{30}H_{48}O_3$	*Trametes odorata*	m.p. 259–261°	(*10*)
Treleasegenic acid	(XD)	$C_{30}H_{48}O_5$	*Lemaireocereus treleasei*	m.p. 280–290°	(*135*)
Tumulosic acid	(CDXXIII)	$C_{31}H_{50}O_4$	*Polyporus* spp.; *P. tumulosus; P. australiensis; Poria cocos* Wolf	$[\alpha]_D^{20}$ +8.1° (CHCl$_3$); m.p. 306°	[(*4*), Vol. III, p. 94; (*5*), p. 841]
Ursolic acid	(ID)	$C_{30}H_{48}O_3$	Wax of leaves and fruits of many plants; *Viscum album; Salvia officinalis; Punica granatum; Cladonia silvatica*	$[\alpha]_D$ +66° (pyridine); m.p. 285–291°	[(*4*), Vol. III, p. 168; (*5*), p. 823]
Uvaol	(D)	$C_{30}H_{50}O_2$	*Crataegus cuneata; Leucothoë keiskei* Miq.; *Arctostaphylos uva-ursi*	$[\alpha]_D$ +69–74° (CHCl$_3$); m.p. 232–233°	[(*4*), Vol. III, p. 168; (*5*), p. 824]
Zeorin	(CDL)	$C_{30}H_{50}O_2$	*Nephroma areticum*	$[\alpha]_D$ +48°–55°; m.p. 228–253°	(*136*)

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CHAPTER 11

Terpenoids: Metabolism *

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I. The "Isoprene Rule" and Chemism of Terpenoid Biosynthesis

The isoprene hypothesis, formulated by Wallach (*1*) and strongly promoted by Ruzicka and co-workers (*2, 3*), states that terpenes and terpenoids are synthesized in nature from isoprene and isopentane units, respectively. The most diverse linkages were proposed for these units. The chemistry of terpenoid biogenesis was advanced, above all, through work on the metabolism of cholesterol. Reference is therefore made to Chapter 3 in this volume, as well as to comprehensive discussions (*2–9*).

Until quite recently, the following acids were recognized as experimentally proved precursors in sterol biogenesis: acetic acid, leucine and valeric acid, β,β-dimethylacrylic acid (DMA), β-methylglutaconic acid, β-hydroxy-β-methylglutaric acid (HMG), β-hydroxyisovaleric acid, and mevaldic acid (MVALD) (see references *6* and *7*, and Chapter 3 in this volume). The discovery of mevalonic acid by Folkers and co-workers (*6, 8*) resulted in the first substantial progress. Through the investigations of the groups headed by Bloch *et al.* (*6*) it became certain that mevalonic acid (VI) was, up to this point, the most important isoprenoid unit. The aforementioned acids proved to be less effective precursors than mevalonic acid. Further interesting investigations by Bloch's and Lynen's groups then yielded proof that the "active isoprene," i.e., the real C_5 unit of all terpenes and terpenoids, is not mevalonic acid itself, but the isopentenyl pyrophosphate (IX) derived therefrom through phosphorylation and decarboxylation (*6, 9–12*). In view of the new data, a comprehensive scheme for the biogenesis of terpenes and terpenoids can be formulated (Fig. 1), based essentially on the work of Lynen (*11–13*).

From this scheme it can be seen that the same precursor, namely

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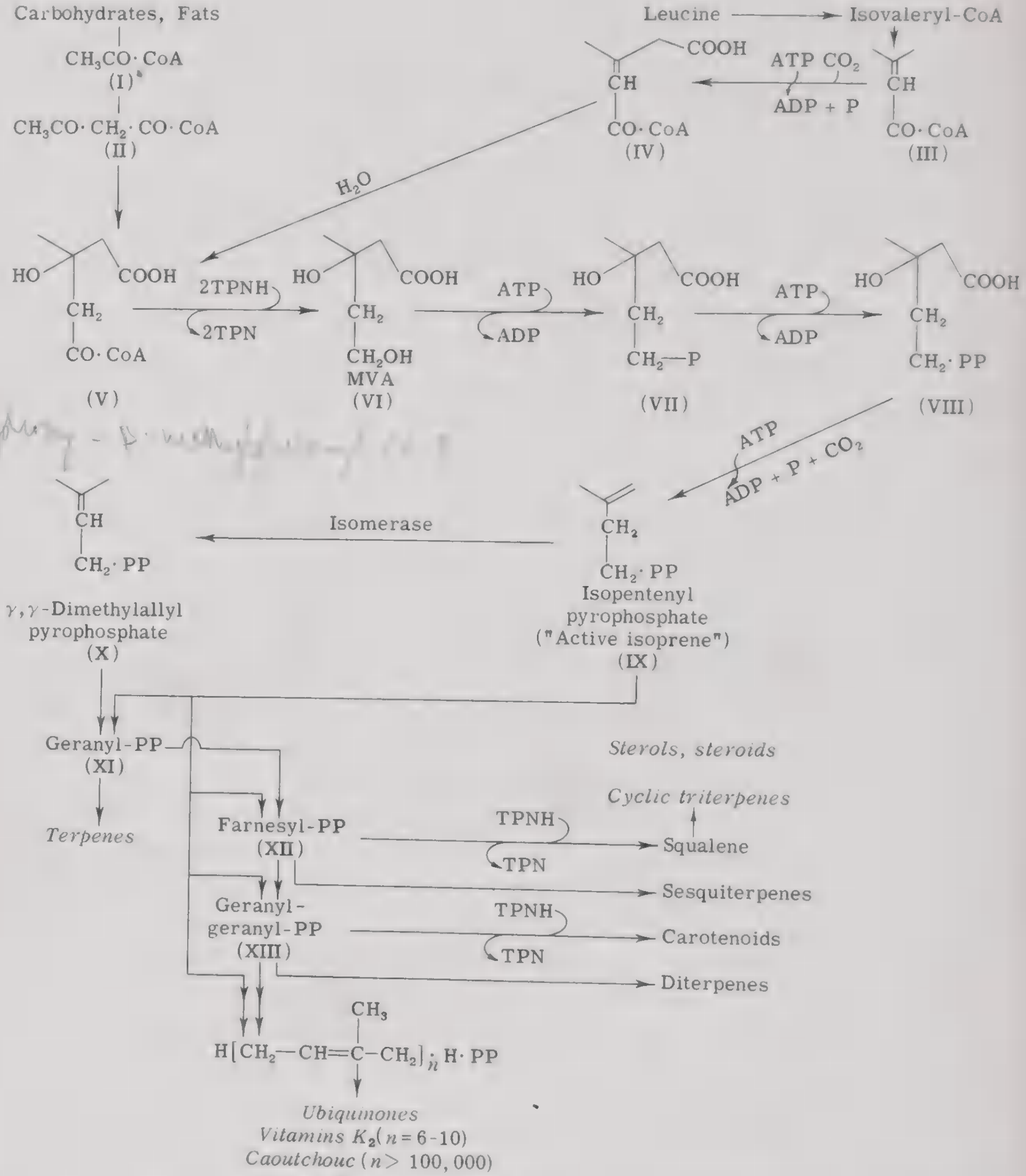
* Translated from the German by Dr. Herbert S. Heineman, University of Pittsburgh School of Medicine.



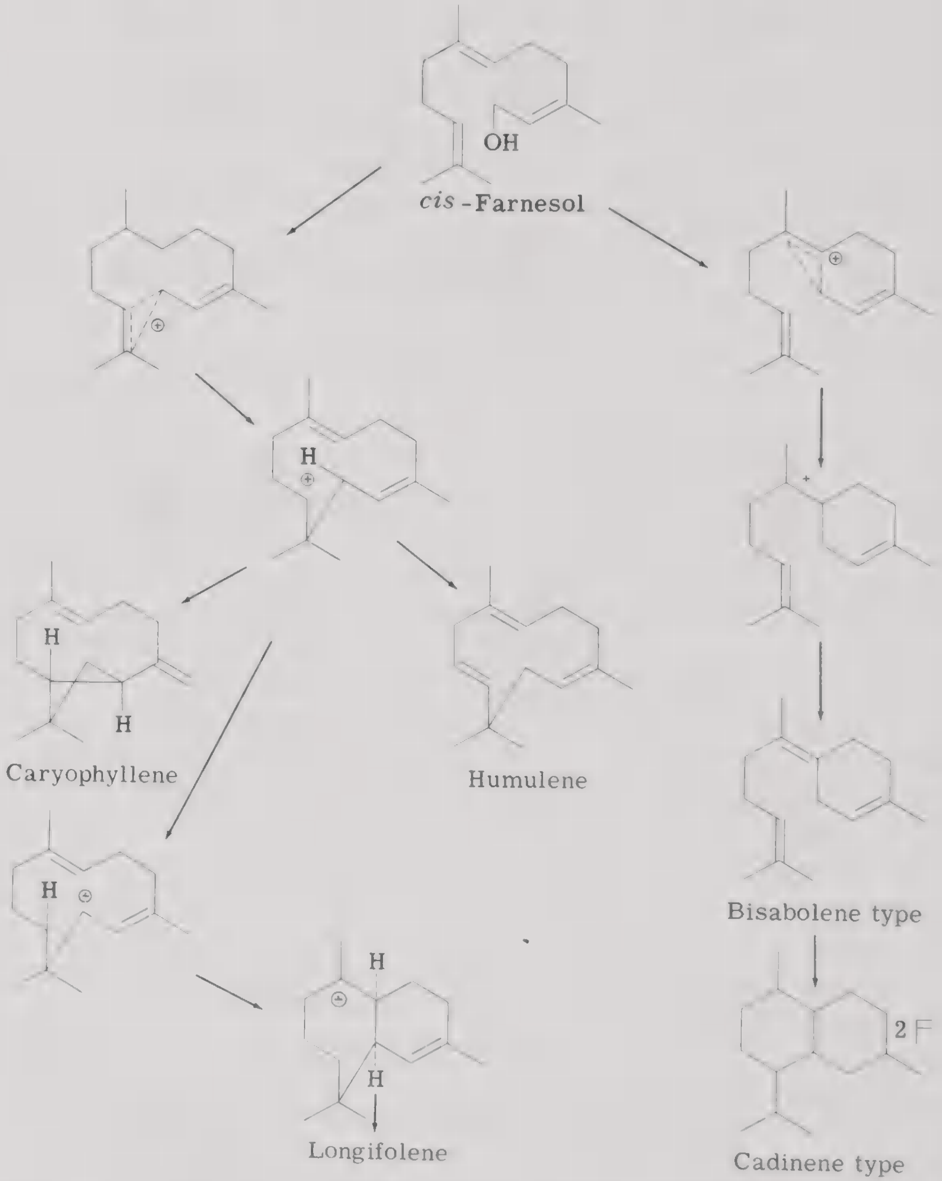
-hydroxy - β - methylglutaryl CoA

FIG. 1. Biogenesis of terpenes and terpenoids. Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; TPN and TPNH, respectively, oxidized and reduced triphosphopyridine nucleotide; CoA, coenzyme A; MVA, mevalonic acid; PP, pyrophosphate.

β-hydroxy-β-methylglutaryl coenzyme A (V), is derived on the one hand from fats and carbohydrates through acetyl coenzyme A (I) and on the other hand from leucine through the coenzyme A compounds of isovaleric acid, β-methylcrotonic acid (=β,β-dimethylacrylic acid) (III), and β-methylglutaconic acid (IV). This precursor, on reduction, yields the well-known mevalonic acid (VI). Phosphorylation with ATP leads to mevalonic acid 5-pyrophosphate (VIII) via the 5-phosphate (VII), and further reaction with ATP, through the loss of CO_2 and phosphoric acid, gives isopentenyl pyrophosphate (IX). The further steps beyond this "active isoprene" are clarified by the interesting results of Lynen (*11–13*). According to these, the isopentenyl pyrophosphate is then isomerized to γ,γ-dimethylallyl pyrophosphate (X) through the enzyme isopentenyl pyrophosphate isomerase. The formation of this compound is important, since the carbonium ion is first represented in the allyl pyrophosphate, whose transformation with the reactive double bond of the isopentenyl pyrophosphate leads to the C—C linkage of the C_5 units. The geranyl pyrophosphate (XI) thus formed can react with a further molecule of isopentenyl pyrophosphate to form farnesyl pyrophosphate (XII). Through elimination of the O—P bond with the help of phosphatase, geranyl pyrophosphate yields geraniol and farnesyl pyrophosphate, farnesol as parent compounds for terpenes and sesquiterpenes, respectively.

Through reduction with TPNH, however, two farnesyl residues can combine to form squalene, this being the biogenetic parent compound of cyclic triterpenes and sterols (cf. Chapter 3, this volume). With the further incorporation of isopentenyl pyrophosphate, farnesyl pyrophosphate is transformed into geranylgeranyl pyrophosphate, which yields diterpene combinations on hydrolysis and carotenoids on reductive dimerization and subsequent dehydration. The further synthesis to polyterpene combinations, solanesol (*14*), terpenoid side chains of ubiquinones, and the K_2 vitamins (*15–19*), as well as caoutchouc, is illustrated in Fig. 1. The validity of this scheme could be demonstrated by the isolation of γ,γ-dimethylallyl alcohol, geraniol, farnesol, and probably geranyl geraniol, as well as the corresponding pyrophosphates (*12*). Thus the "biogenetic isoprene rule" of Ruzicka (*2–4*) is confirmed, according to which terpenes and terpenoids are derived from the corresponding regularly constructed aliphatic parent compounds (geraniol, farnesol, geranylgeraniol, squalene).

Recognition of the "active isoprene" and its precursors makes it possible, through the introduction of tagged intermediate products, to obtain a clear picture of the biosynthetic pathway of terpene compounds. Thus, isopentenyl pyrophosphate can be synthesized (*11*) and, within the organism, converted into terpene compounds, e.g., through enzymes of the liver into cholesterol (*11*), or in the latex of *Taraxacum kok-saghyz* (Arreguin, according to reference *11*) into radioactive caoutchouc; the use of radioactive mevalonic acid, however, is much simpler. This precursor is converted to terpene compounds so quickly that one might practically speak in terms of incorporation en bloc. Through decomposition of the radioactive compound the position of the radioactive carbon atoms can be determined and conclusions be drawn as to whether a terpene compound arises from a regular sequence of linkages, i.e., head-to-tail. β,β-Dimethylacrylic acid can, in some cases, likewise be incorporated in a more or less unaltered form (en bloc) (*20, 21*); in other cases, however, it undergoes so much degradation to acetic acid before its incorporation that it does not seem suitable for biogenetic



studies (*22*). With 2-C^{14}-mevalonic acid, various terpene compounds were obtained *in vivo*, e.g., cineol in *Eucalyptus globulus* Lab. (*23*), α-pinene in *Pinus attenuata* (*24*), caoutchouc in *Ficus elastica* (*25*) and teak (*26*).

Far more interesting is the incorporation into irregularly constructed

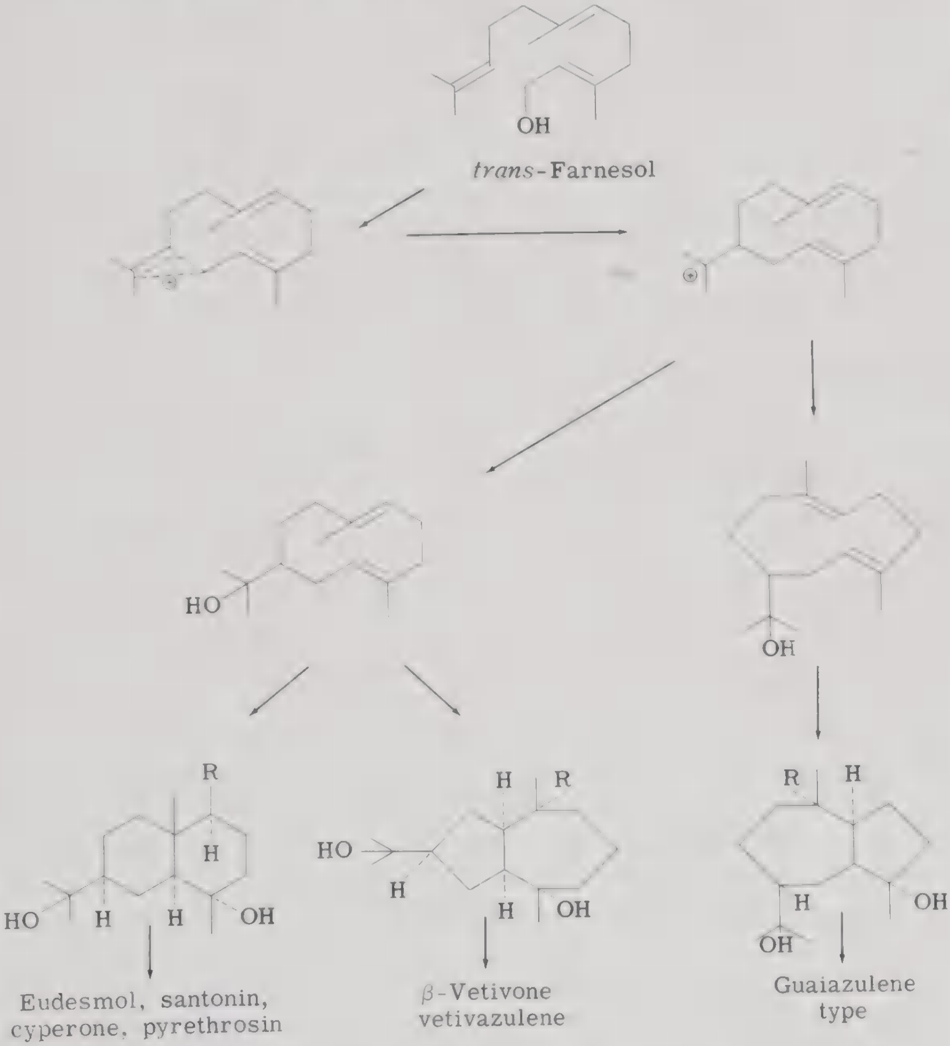
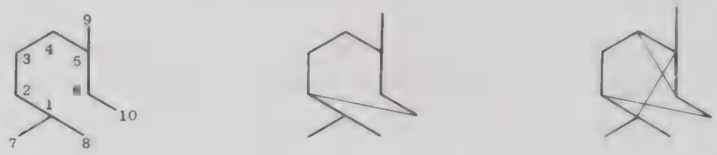


FIG. 2. Biogenesis of sesquiterpenes (*28*).

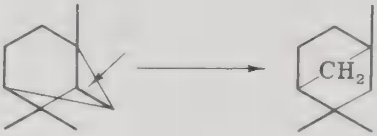
terpenes and terpenoids. These cases are considered below or in Chapter 3 of this volume. With radioactive tagged β,β-dimethylacrylic acid the biogenesis of pulegone was elucidated in *Mentha pulegium* L. (*21*). A surprising finding was that latex from *Hevea brasiliensis* yielded *in vitro* active caoutchouc from tagged acetic acid or DMA (*27*).



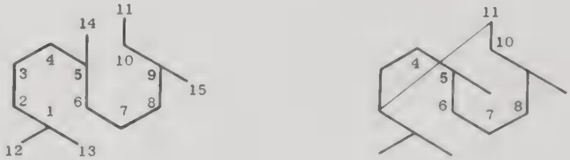
Geraniol
1,6-Cyclocitral
2,6-Nepetalactone

Dipentene
1,6-Pinene
1,5-Borneol
1,10-Carene
2,4-Sabinene

Teresantalol

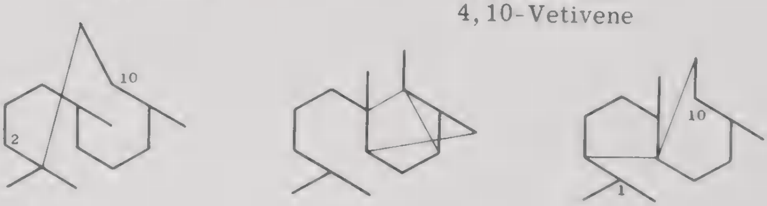


Fenchol



Farnesol
6,11-Bisabolene
1,6-Iresin
5,10-Iresin

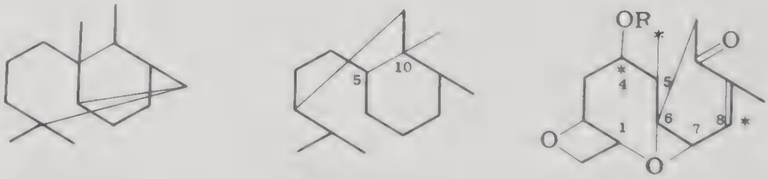
Aristolactone
6,11-Cadinene
5,10-Selinene
6,10-Guaiene
4,10-Vetivene



Humulene
2,10-Caryophyllene

α-Santālene

Acorone
1,10-Cedrene



Longifolene

Eremophilone

Tricothecin

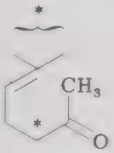
FIG. 3. Biogenetic formulation for terpenes and sesquiterpenes. The numbers indicate additional bonds.

II. Ring Closures and Rearrangements

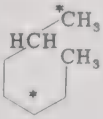
According to Ruzicka's biogenetic "isoprene rule," which has been confirmed by the results of Bloch as well as Lynen, primarily geraniol, farnesol, and geranylgeraniol are formed. In the biologic system, according to fundamental considerations of Ruzicka and co-workers, especially Eschenmoser (*2–5*), these aliphatic parent compounds are folded, under the influence of cyclizing ferments, from their structural equilibrium in the direction of a form that is thermodynamically stable and suited for coupled cyclization. Thereupon ring closure takes place with the help of an ionic or radical mechanism.

The relationships for sesquiterpenes are illustrated in Fig. 2 (*28*).

Similar to ring closures, rearrangements are also stereospecific (*28*). Certain enzyme systems produce folded forms optimal for Wagner's rearrangements, 1,2-migrations of methyl groups and H atoms, as well as H eliminations. In the irregularly constructed terpene compounds thus obtained, the originally regular structure is often barely recognizable. This is even more likely when the biogenetic formulation is used rather than the classic one. This is evident from Fig. 3, in which all the important ring systems of the terpenes and sesquiterpenes have been fitted into the biogenetic basic structure without regard to very important steric relationships. In this manner of writing, the biogenesis of tricothecin is more clearly illustrated than in the usual formulation (Chapter 3, this volume). Experiments with 2-C^{14}-mevalonic acid had shown that the methyl group on C-5 is radioactive (*29*). This finding can be explained only by two methyl group migrations, namely the original inactive methyl group from 5 to 6 and the active geminate one from 1 to 5 (*29*). Many statements regarding the biogenesis of terpenes and sesquiterpenes are for the present still hypothetical, e.g., carquejol (*30*), thujic acid and thujaplicin (*31*). According to another hypothesis, the *n*-heptane occurring to an extent of 95% in the "terpentene oil" of *Pinus jeffreyi* and *Pinus sabiniana* has a terpenoid origin. Thus it could be formed from a degradation product such as methylheptenone, common in plants, through reduction and oxidative demethylation (XIV, XV). Investigations with 2-C^{14}-mevalonic acid in *Pinus jeffreyi* showed,



(XIV)



(XV)

however, that the heptane was inactive. It does not, therefore, have a terpenoid origin (*26*).

Approximately one-third of all diterpenes including cyclized compounds such as dextropimaric acid have the primary carbon skeleton of geranylgeraniol. In other cases, new diterpene skeletons have arisen by means of migration of methyl groups, contraction and expansion of

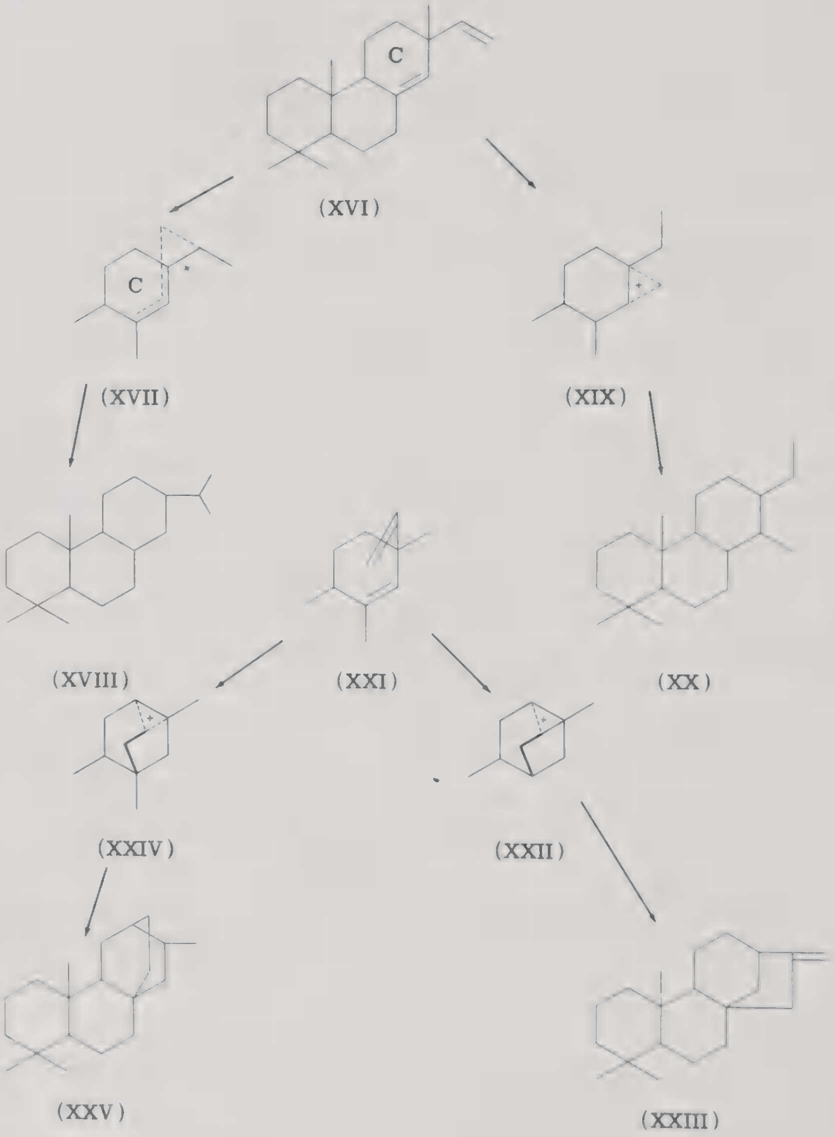
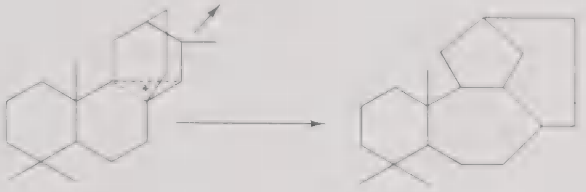


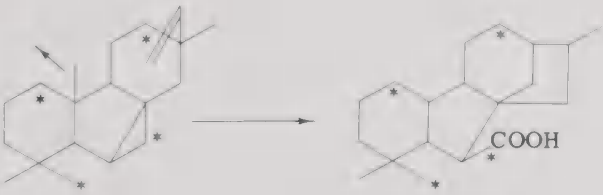
FIG. 4. Rearrangements of the carbon skeleton in the biogenesis of diterpenes.

rings, and other rearrangements. These are distinguished principally in ring C.

The resin acids of pine, such as dextropimaric acid and abietic acid, according to a hypothesis of Sandermann (*33*) and Hasselstrom (*34*) as well as of Ruzicka (*2*) and Wenkert (*35*), are formed from a common parent substance (XVI–XVIII) (cf. Fig. 4). This theory has meanwhile been confirmed in *Pinus sylvestris* L. with the use of tagged dimethylacrylic acid (*36*). The carbon skeleton of vinhaticoic acid (XX) could be formed from the same basic skeleton by way of a cyclic carbonium ion (XIX). According to a very interesting theory by Wenkert (*35*), the skeleton of phyllocladene (XXIII) is formed from the cation (XXII) and that of atisine (XXV) from the cation (XXIV). The latter cation can be formed from (XII) through a hydride shift (*35, 38*). In the formation of the diterpene skeleton of the alkaloid lycoctonine, oxidative demethylation takes place in ring C together with simultaneous expansion of ring B and contraction of ring C (XXVI, XXVII) (*37*).



(XXVI) (XXVII)



(XXVIII) (XXIX)

The biogenesis of gibberellic acid (skeleton = XXIX) was elucidated by Birch and Smith working with tagged acetic acid and mevalonic acid (*38*). Since the carboxyl group is active, the acid could have been formed from a precursor (XXVIII). Rosenonolactone also was synthesized from tagged mevalonic acid (*38*). It was thereby found that from the *two* geminate C atoms on ring A the methyl group was active and the *carboxyl* group inactive. This shows that oxidation in ring A proceeds in a strictly sterospecific manner. Methyl group migrations as in rosenonolactone (*38*) or columbin (*39*) are easily explained as 1,2-migrations. Even for the isopropyl group of totarol a similar mechanism can be postulated. Wenkert assumes an intermediate spiran compound, from which totarol and also podocarpic acid are formed (*35*).

The biogenesis of the triterpenes was elucidated above all by Ruzicka's group of investigators (*2–5*). Stork and Burgstahler independently arrived at the same results (*40*). The parent compound of all triterpenes is all-*trans*-squalene, which, with the help of enzymes, undergoes a specific manner of folding. This enzymatic folding is the prerequisite for a subsequent nonstop reaction; thereby, a sequence of stereospecific 1,2-rearrangements of hydrogen atoms and methyl groups takes place, with the final loss of a proton (or addition of an anion). In this manner, for example, by means of chair-chair-chair-boat-boat (CCCBB) folding, a lupanyl cation arises, from which through appropriate rearrangements the known types of pentacyclic terpenes are formed. The various foldings in the biogenesis are seen in Fig. 5. The

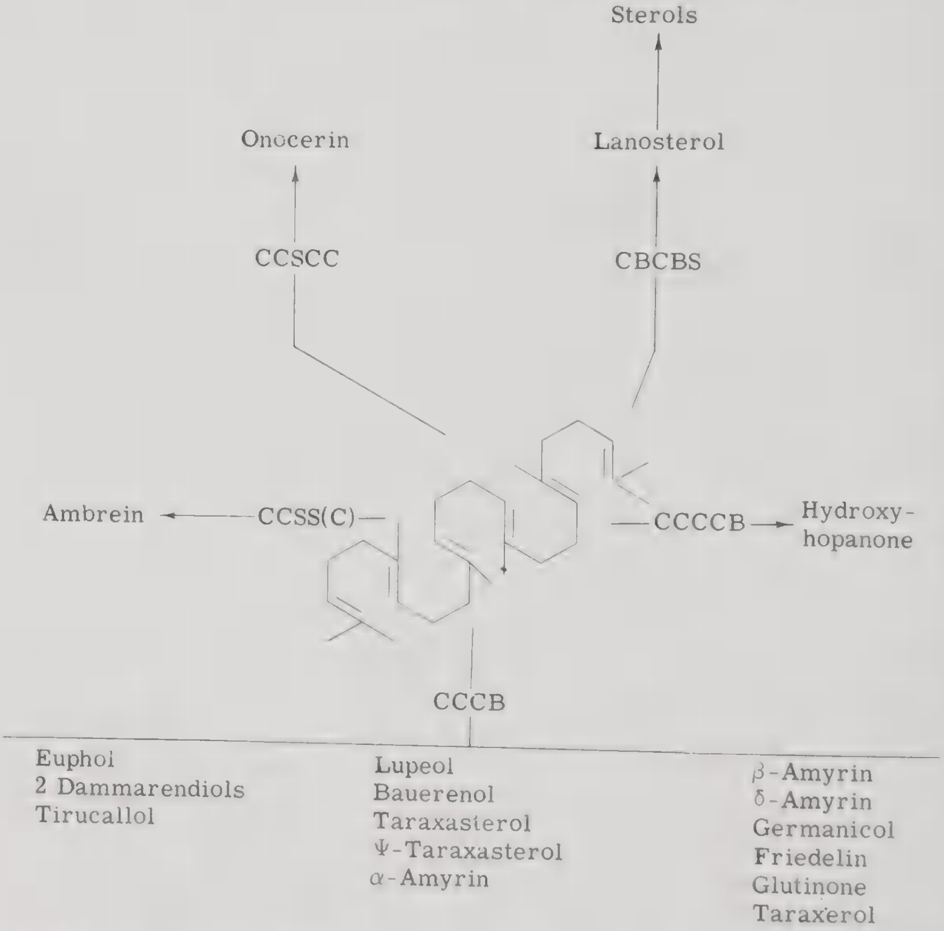


FIG. 5. Biogenetic folding of the rings in the triterpenes. *C* = chair form; *B* = boat form; *S* = straight form.

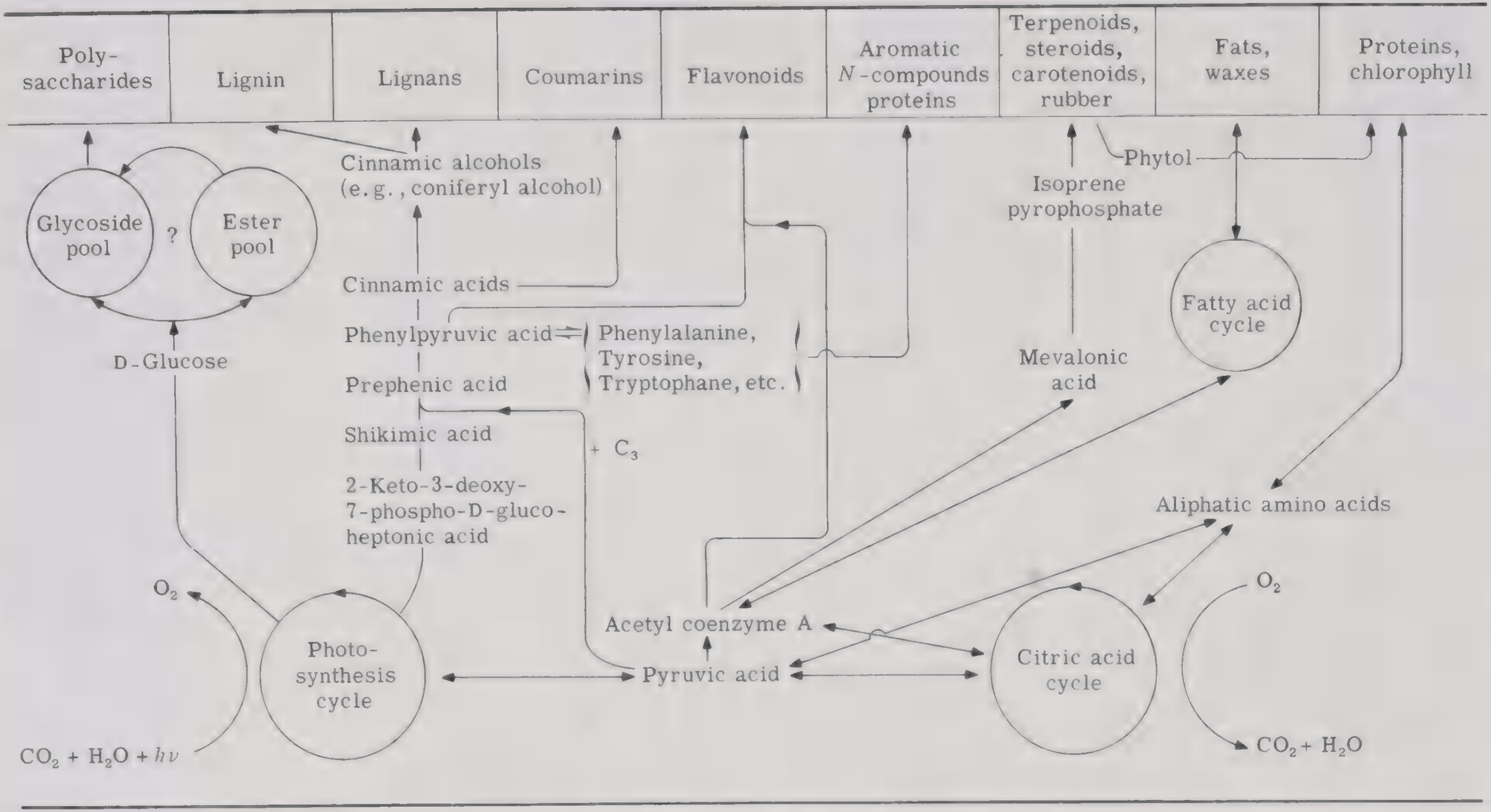


FIG. 6. Biosynthesis of plant materials.

biogenesis of lanosterol has been especially well explored, since this triterpene is an intermediate in the biogenesis of sterols from squalene (Chapter 3, this volume). Biogenetic investigations with tagged substances were carried out on eburicoic acid (*41, 42*) and soysapogenols (*43*). It was found that the reactions leading to triterpene are sterically strictly controlled. With this, the biogenetic isoprene rule also receives experimental support.

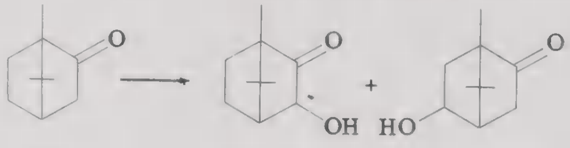
Interesting is a new type of triterpene with open ring A, e.g., in dammarenolic acid and nyctanthic acid. It is possible that this type arises through the cleavage of a ketone (*44*).

The position of the terpenoids in the framework of the total plant biogenesis is shown in Fig. 6.

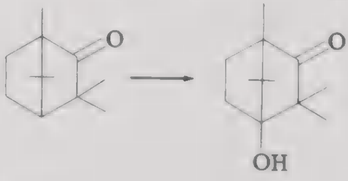
III. Biochemical Oxidations and Hydrogenations

The aliphatic primary products of terpene biogenesis—geraniol, farnesol, geranylgeraniol, and squalene—as well as the combinations arising therefrom by cyclization, can undergo many changes through oxidation and reduction. The exact pathway of these biogenetic reactions has, until recently, been elucidated only in a few cases.

Introduction of an OH group can be accomplished through direct oxidation, through reduction of a keto or an aldehyde group, and through hydration of an ethylene bond. Thus, for example, terpineol is formed by hydration of limonene. That hydroxyl groups can be directly introduced into terpene compounds *in vivo* was demonstrated by feeding experiments with camphor (XXX) in dogs (*45*): 3-Oxocamphor (XXXI) and 5-oxycamphor (XXXII) were isolated. Dimethylcamphor (XXXIII) under similar conditions gave the hydroxy compound (XXXIV) (*46*).



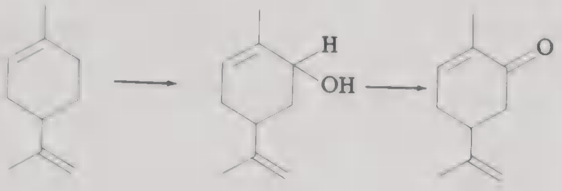
(XXX) (XXXI) (XXXII)



(XXXIII) (XXXIV)

It is assumed that the plant possesses a similar capacity for hydroxylation. Fungi are known to be able to introduce OH groups into sterols, predominantly in positions 6β, 11α, 11β, and 17α (*47*).

One of the most important problems in the oxidation of terpenes is the formation of hydroxyl and keto groups in the vicinity of ethylene bonds. Thus in *Carum carvi* the biogenesis of carvone (XXXVII) from limonene (XXXV) is thought to proceed by way of carveol (XXXVI)



(XXXV) (XXXVI) (XXXVII)

(*48*). This hypothesis is supported by the fact that, to begin with, particularly limonene is formed in plants, even more so carveol, and above all carvone. Kalitzki (*49*) and Ihloff (*50*), in their work on the biogenesis of carvone in *Anethum graveolens*, also agreed with this hypothesis. Ihloff suggested the following reaction sequence:

Unknown precursor → Phellandrene → Limonene → Carvone

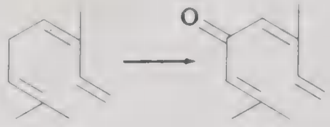
A further very detailed investigation on the part of Luyendijk (*51*) on the biogenesis of carvone in *Carum carvi* and *Anethum graveolens* could not refute the above hypothesis (*48*). Two additional suggestions, which picture a hypothetical "key terpene" as immediate precursor for carvone or carveol, have meanwhile been superseded by the clarification of the biogenesis up to geraniol. The role of limonene as precursor is also supported by the finding that it forms carveol and carvone by autoxidation (*52*). Furthermore, the simultaneous occurrence of carvone and limonene in many oils points to the biogenetic connection between the two compounds (Table I).

TABLE I
THE SIMULTANEOUS OCCURRENCE OF CARVONE AND LIMONENE

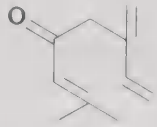
Species	Carvone	Limonene	Dipentene	Carveol	Dihydrocarvone	Dihydrocarveol
Carum carvi	*α*, +	*d*, +	+	+	+	+
Mentha spicata	*l*, +	*l*, +	+			
Cymbopogon martini Stapf var. Sofia	*dl*, +	+	+			
Lippia spp.	*l*, +	*l*, +	+			
Anethum graveolens L.	+	*d*, +	+		+	

The biogenesis of many other terpene derivatives can be explained by the same oxidation chemism. Thus ocimenone (XXXIX) is formed from ocimene (XXXVIII). Both compounds are found in *Lippia asperifolia* Rich together with myrcenone (XL), which is certainly formed in a similar manner (*53*).

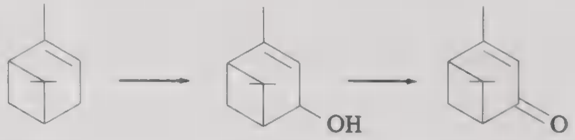
In *Boswellia carteri* Birdw. (oil of olibanum) α-pinene (XLI), verbenol (XLII), and verbenone (XLIII) occur together (*54*). This suggests the biogenetic pathway (XLI–XLIII).



(XXXVIII) (XXXIX)



(XL)



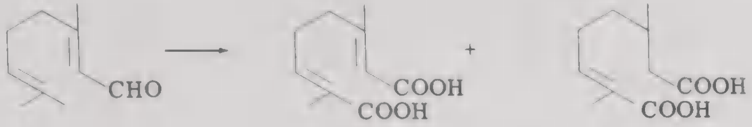
(XLI) (XLII) (XLIII)

The following compounds probably are formed in a similar manner: pinocarveol and pinocarvone (both in *Eucalyptus globulus*), sabinol, piperitenone, α-atlantone, turmerone, β-cyperone, artemisin, hinokiol, and hinokione. As shown below, many more terpene derivatives are formed from this type of hydroxy and keto terpenoid through biologic hydrogenation.

One of the most interesting biochemical reactions is "methyl group oxidation." Such oxidation of aromatic compounds in the animal body, e.g., from toluene to benzoic acid, has been known for a long time. Methyl group oxidation to the carboxyl group goes through the following stages:

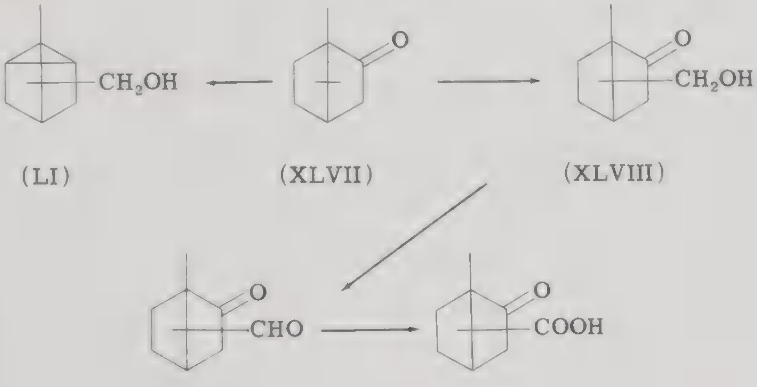
$$—CH_3 \rightarrow CH_2OH \rightarrow —CHO \rightarrow —COOH$$

Kuhn and associates (*55*) developed concepts with regard to the chemism of the individual steps. By injection of citral (XLIV) into dogs, Kuhn and Livida (*56*) obtained "Hildebrandt acid" (XLV) and some "dihydro-Hildebrandt acid" (XLVI). Asahina and Ishidate, after feeding camphor (XLVII) to dogs, obtained all the predicted members of the methyl group oxidation sequence, namely the alcohol (XLVIII), aldehyde (IL), and acid (L) (57). Moreover, reduction yielded teresantol (LI).

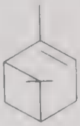


(XLIV) (XLV) (XLVI)

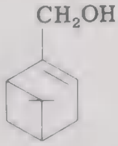
It is apparent from a comparison of the occurrence of numerous terpene compounds that methyl group oxidation plays an important role in plants also. As an illustrative example, the methyl group oxidation of α-pinene is presented (XLI–LIV). Of these compounds, *d*-α-pinene (XLI), *d*-myrtenol (LII), and *d*-myrtenal (LIII) occur in *Eucalyptus globulus* (58) and myrtenic acid (LIV), by contrast, in



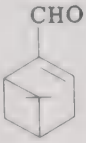
(IL) (L)



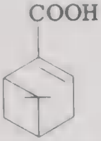
(XLI)



(LII)



(LIII)



(LIV)

Chamaecyparis formosensis. Examples of other plants that display several steps in methyl group oxidation are *Andropogonis citrati* (citronellol, citronellal, and citronellic acid), *Citrus limonium* (Linn.) Burm. f. (geraniol, citral, geranic acid), *Santalum album* L. (santalol and santalic acid), and *Pinus* spp. (dextropimarinal and dextropimaric acid).

TABLE II

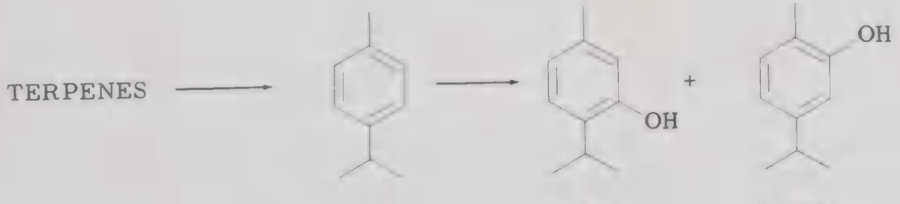
STEPS IN METHYL GROUP OXIDATION

	$—CH_3$	$—CH_2OH$	—CHO	—COOH	—H
Monoterpenes		Geraniol	Citral	Geranic acid	
		Citronellol	Citronellal	Citronellic acid	
	α-Pinene	Myrtenol	Myrtenal	Myrtenic acid	
	Monocyclic terpenes	Perillyl alcohol	Perillaldehyde		
			Phellandral	Phellandric acid	Santene
Sesquiterpenes	Cadinene	Santalol	Lactaroviolin	Santalic acid,	—
	Selinene, etc.	Lanceol		Atlantolactone	
		Agarol		Guaiazulenic acid	
		Sesquibenihiol		Group of sesquiterpene lactones	
Diterpenes	Manool	Elliotinol	Dextropimarinal	Group of resin acids,	Fichtelite
	Sclareol	Andrographolide	Isodextropimarinal		
	Rimunene			Columbin,	
	Ferruginol etc.			Gibberellic acid	
Triterpenes (CH_3 group on C_4)	Most triterpenes, e.g., Betulin, Lupeol, β-Amyrin	Barringtogenol, Hederagenin, Bassic acid, Soysapogenol, Terminolic acid Arjunolic acid	Quillaic acid	Barringtogenolic acid, Medicagenic acid, α-Boswellic acid	Cycloeucalenol, Citrostadienol, Lophenol

It can be seen in Table II that the aldehyde step occurs predominantly among monoterpenes whereas the carboxyl step occurs among sesqui-, di-, and triterpenes: In certain cases the carboxyl group may be split off, thus achieving complete demethylation. Elimination of the three methyl groups of squalene always occurs in the biogenesis of sterols. However, a number of demethyltriterpenes are known as intermediates in which only one methyl group has been removed, e.g., lophenol.

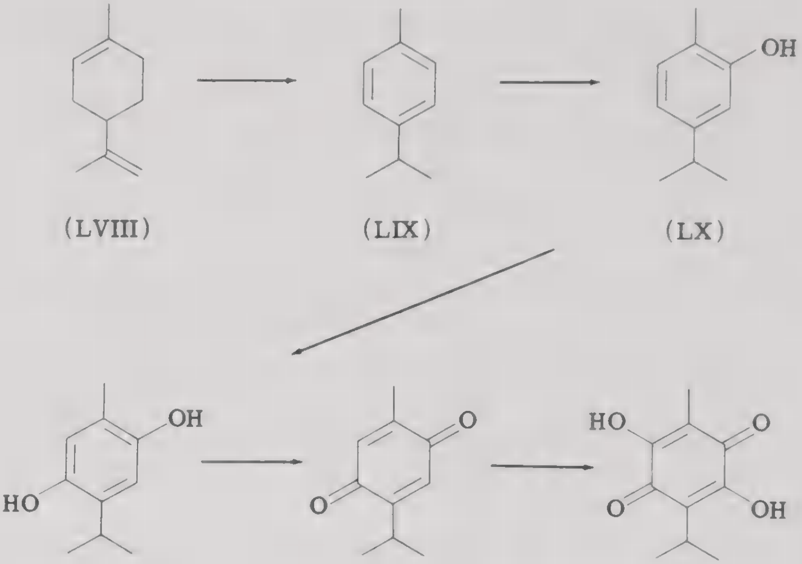
Oxidation reactions also include dehydrogenations. Examples of compounds arising in this manner are cosmene, safranol, cymol, *ar*-turmerone, calamenene, azulene, dehydroabietic acid, vitamin A, and tanshinone. Numerous phenols should also be mentioned here, such as carvocrol, thymol, the thujaplicins, thymohydroquinone and its derivatives, ferruginol, hinokiol, totarol, nimbiol, podocarpic acid, xanthoperol, and gossypol.

The formation of phenols can take place by direct oxidation of an aromatic ring. Thus phenylpyruvic acid is converted in the animal to 3,4-dihydroxyphenylalanine (the precursor of adrenaline) and in the plant to coniferyl alcohol (the precursor of lignin). In each case, the benzene ring has acquired two phenol groups through oxidation. Aromatic compounds of terpenoid origin can also form phenols in this manner. Thus, in *Carum copticum* (oil of ajowan), the thymol content increases with the accumulation of oil and continues to increase after the maximum oil content has been reached (*59*, *60*). According to this, thymol should be formed from *p*-cymol, which has been demonstrated in the oil. The biosynthetic pathway would then be as shown (LV–LVII).



(LV) (LVI) (LVII)

The oil of *Monarda fistulosa* ("wild bergamot" oil) contains, in addition to limonene (LVIII), a whole sequence of aromatic compounds, *p*-cymol (LIX), carvacrol (LX), thymohydroquinone (LXI), thymoquinone (LXII), and dihydroxythymoquinone (LXIII), the biogenesis of which can be represented by the sequence of formulas (LVIII–LXIII).

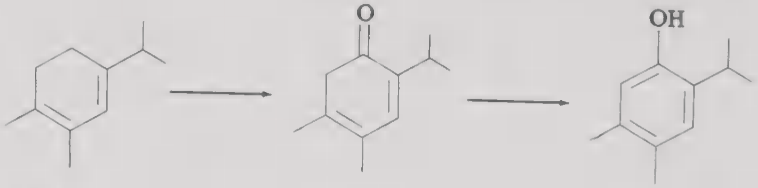


(LVIII) (LIX) (LX)

(LXI) (LXII) (LXIII)

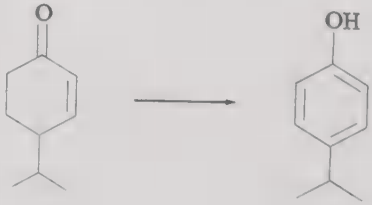
Libocedrus decurrens also contains phenolic compounds, namely thymoquinone, thymohydroquinone, libocedral, 3-libocedroxythymoquinone, as well as β- and γ-thujaplicin.

It is also conceivable that a phenol group is formed by enolization of a keto group, approximately as shown in the sequence (LXIV–LXVI).



(LXIV) (LXV) (LXVI)

There is good reason to believe that this pathway is the preferred one among many diterpene phenols. Even the formation of *p*-isopropylphenol (LXVIII) in *Eucalyptus cneroifolia* can result in this manner



(LXVII) (LXVIII)

from a precursor that could arise from cryptone (LXVII), which occurs in this oil.

According to Erdtman, carvacrol is formed in a similar fashion from carone in *Libocedrus decurrens* (*61*). Carone, furthermore, would be the key compound in the biogenesis of the three thujaplicins, thujic acid, chamic acid, and chaminic acid (Fig. 7).

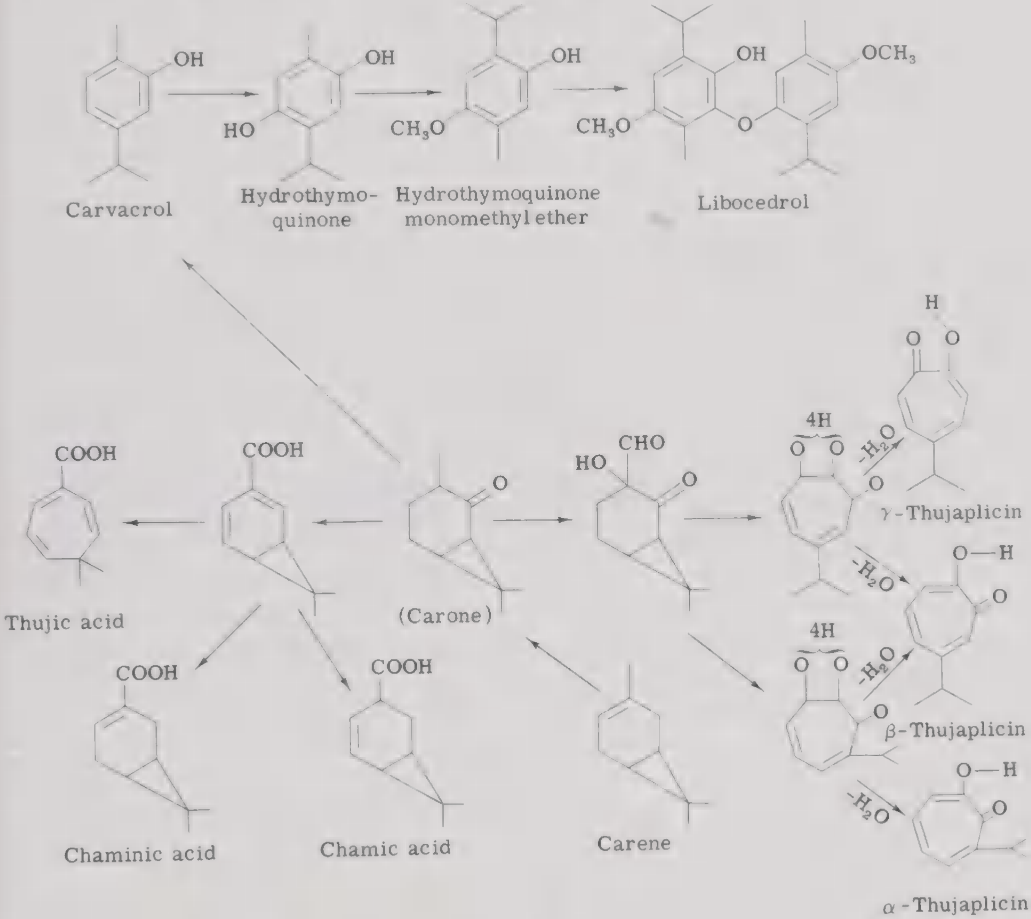
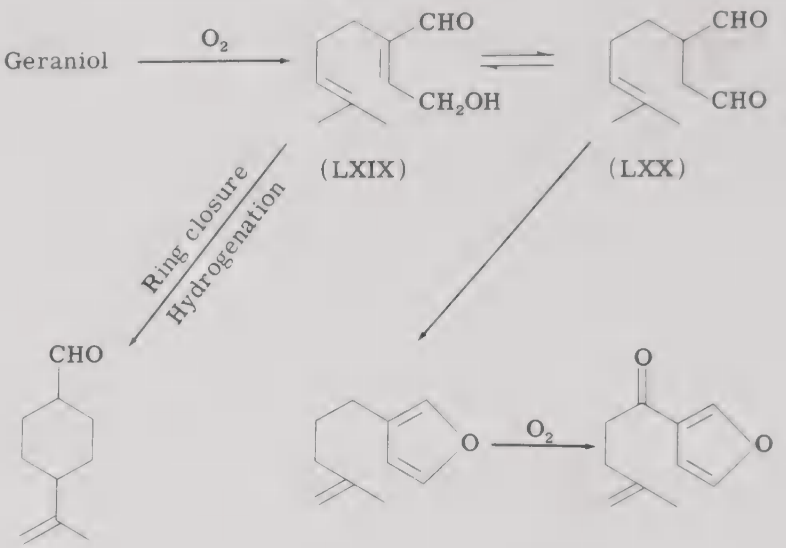


FIG. 7. Carone as the key compound in the biogenesis of terpenoid compounds. After Erdtman (*61*).

Up to the present time, no biogenetic pathways have been described for the formation of numerous terpene compounds with the furan ring (examples: perillene, menthofuran, dendrolasin, linderene, columbin, and vuocapenic acid). It may be assumed that these furans are formed from 1,4-dialdehydes or ketoaldehydes. For the biogenesis of perillaldehyde (LXXI), perillene (LXXII), and perillyl ketone (LXXIII) in *Perilla* spp., sequence (LXIX–LXXIII) is suggested.



(LXIX) (LXX)

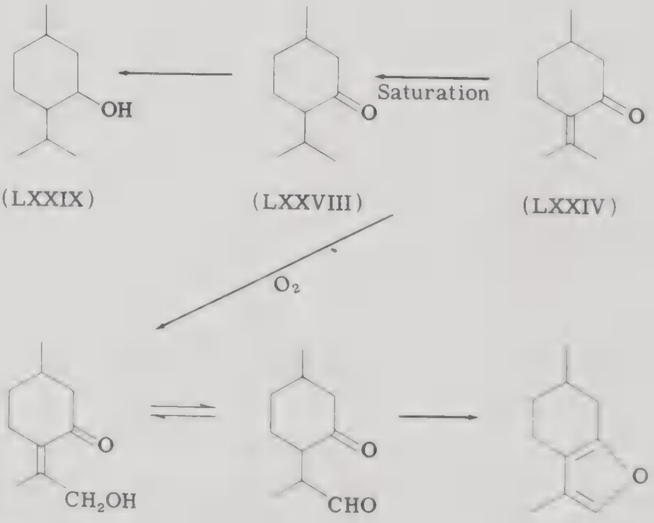
(LXXI) (LXXII) (LXXIII)

In *Perilla nankinensis*

In *Perilla citriodora*

In *Perilla frutescens* Brit.

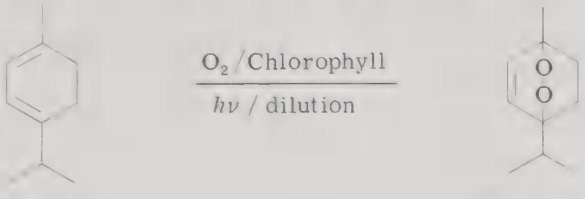
In *Mentha.* spp. the formation of menthofuran (LXXVII) could proceed from pulegone (LXXIV), which could also be the precursor for menthone (LXXVIII) and menthol (LXXIX).



(LXXIX) (LXXVIII) (LXXIV)

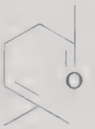
(LXXV) (LXXVI) (LXXVII)

Saturated oxides, such as cineol, can be formed by splitting off water between two hydroxyl groups. A special situation is the biogenesis of ascaridol (LXXXI) in *Chenopodium anthelminticum*. Schenck recognized the fact that this compound is formed from α-terpinene (LXXX) by photochemical addition of O_2, activated by chlorophyll (*62*). This diene reaction with oxygen was to a large extent carried out *in vitro*.

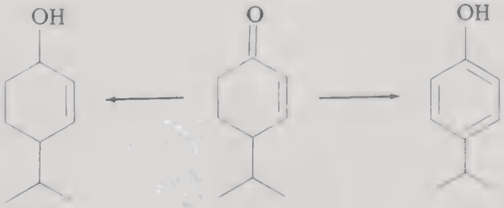


(LXXX) (LXXXI)

In certain cases oxidation can lead to compounds very rich in oxygen (examples: verbenalol, lactucin, picrotoxinin, columbin, terminolic acid). On the other hand, oxidation can, through loss of carbon atoms, lead to compounds whose relationship to terpenes is often unrecognizable. Thus methylheptenone (LXXXII) is found in the essential oil of many plants (*Andropogon citratus, Ocimum canum, Artemisia scoparia,* etc.). As this degradation product occurs in association with aliphatic terpene alcohols and aldehydes, it is presumed to arise through their degradation. In the oil of *Phellandricum aquaticum* L. Striegler found the two degradation products cryptone (LXXXIV) and cryptol (LXXXIII) (*63*). Cryptone occurs also in association with australol (LXXXV) in certain *Eucalyptus* species. It may be assumed that cryptone leads to australol through dehydrogenation and to cryptol through saturation (LXXXIII–LXXXV).



(LXXXII)



(LXXXIII) (LXXXIV) (LXXXV)

According to a hypothesis by Aneja, Mukerjee, and Seshadri (*64*), very extensive degradation of prenyl compounds of structure (LXXVI) leads to unsubstituted furans (LXXXVIII). In favor of this theory is the frequent simultaneous occurrence of compounds which, besides the furan ring (C_2), contain also the prenyl skeleton (C_5) or other terpenoid residues (C_{10}, C_{15}) (Table III).

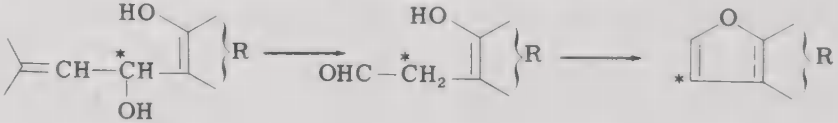
The correctness of this hypothesis can be tested with the help of

TABLE III

OCCURRENCE OF FURANIC COMPOUNDS (C_2) WITH TERPENOID CHAINS (C_5–C_{15})

Family	Species	C_2	C_5	C_2–C_5	C_{10}	C_{15}
Umbelliferae	*Angelica archangelica*	+	+	+		+
	Imperatoria ostruthium		+	+	+	
	Ammi visnaga	+	+			
Rutaceae	*Xanthoxylum flavum*	+	+			
	Xanthoxylum rhetsa	+	+			
	Skimmia japonica	+	+			
	Aegle marmelos	+	+	+	+	
	Casimiroa edulis	+				
	Flindersia species	+	+		+	
Leguminosae	*Derris elliptica*	+	+			
	Pachyrrhizus erosus	+	+			

mevalonic acid tagged at C-6 (*65*). In the furans thus formed the C atom marked in (LXXXVIII) should be radioactive.



(LXXXVI) (LXXXVII) (LXXXVIII)

Not only oxidation, but also hydrogenation leads to secondary alterations in terpene compounds. Biologic hydrogenations with budding yeasts or bacteria have led to the conclusion that the following transformations are possible in this manner (*66*):

(1) *Primary alcohols*

$$R \cdot CH{:}CH \cdot CH_2OH \rightarrow R \cdot CH_2 \cdot CH_2 \cdot CH_2OH$$

(2) *α,β-Unsaturated aldehydes*

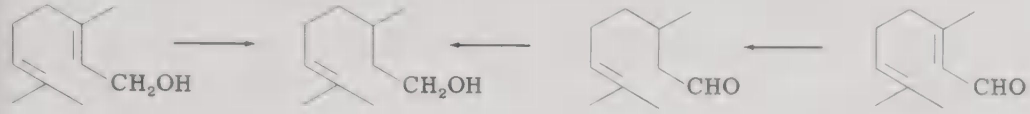
$$R \cdot CH{:}CH \cdot CHO \rightarrow R \cdot CH_2 \cdot CH_2 \cdot CHO \rightarrow R \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot OH$$

(3) *α,β-Unsaturated ketones*

$$\begin{array}{l} R \cdot CH{:}CH \cdot CO \cdot R' \rightarrow R \cdot CH_2 \cdot CH_2 \cdot CO \cdot R' \rightarrow R \cdot CH_2 \cdot CH_2 \cdot CH(OH) \cdot R' \\ \qquad\quad \downarrow \\ R \cdot CH{:}CH \cdot CH(OH) \cdot R' \end{array}$$

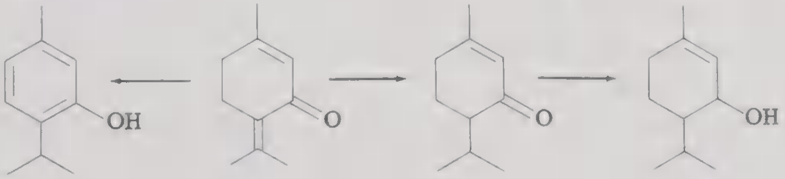
Ethylene bonds thus can be hydrogenated only when they are present in the α,β-position in relation to a keto, aldehyde, or primary alcohol group. In a secondary alcohol, an ethylene bond in the α,β-position is not hydrogenated.

These reactions also play a great part in the biogenesis of many terpene compounds. Thus citronellol (XC) could be formed by hydrogenation of geraniol (LXXXIX) or of citral (XCII) by way of citronellal (XCI). Hydrogenation plays a large role in *Mentha* species. A reaction sequence can lead from pulegone (LXXIV) to menthone (LXXVIII) and menthol (LXXIX).

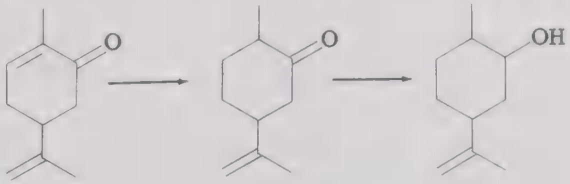


(LXXXIX) (XC) (XCI) (XCII)

The reaction could also be carried out with yeast (*66*). Furthermore, this assumption is in agreement with the observation of Kleber (*67*) and others that, during ripening of *Mentha* species, menthone decreases and menthol increases. Bacon, Jenison, and Kremers assume a menthenone as precursor of menthone (*68*). This assumption is in accord with the above sequence of formulas. Besides pulegone, piperitenone (XCIV), and piperitone (XCV) occur as hydrogenable ketones in *Mentha* species. Through hydrogenation, (XCIV) can be converted into (XCV) and further into piperitol (XCVI). The stage of hydrogenation (XCVI) (piperitol) is not achieved in *Mentha* spp., but it is achieved in *Eucalyptus* spp., e.g., *E. dives*, *E. radiata*, etc. The ethylene bond in piperitol is not hydrogenable, since in (XCVI) one is dealing with a secondary alcohol. The formation of hydrogenated compounds [dihydrocarvone (XCVIII) and dihydrocarveol (IC)] from carvone (XCVII) in the oil of *Carum carvi* can also be easily illustrated (XCVII–IC). In

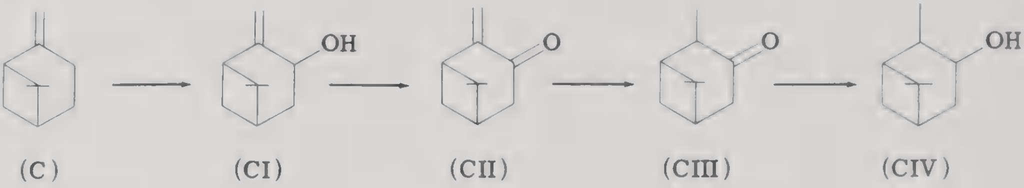


(XCIII) (XCIV) (XCV) (XCVI)



(XCVII) (XCVIII) (IC)

similar fashion, hydrogenation of *l*-pinocamphone (CIII) to pinocampheol (CIV) takes place in the oil of *Hyssopus officinalis* L. The hydrogenated starting compound *l*-pinocarvone (CII), however, is found not in this oil but rather that of *Eucalyptus globulus*. The progression from β-pinene (C) to pinocampheol involves first oxidation and then hydrogenation (C–CIV).



The biogenesis of phytol shows that another possibility for hydrogenation is available to the plant, which is not limited as is the type of hydrogenation mentioned.

IV. Chemotaxonomy

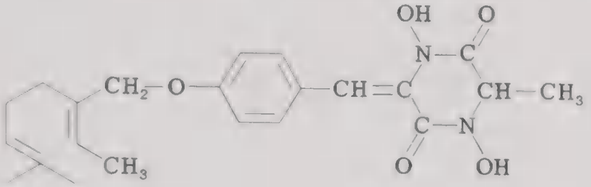
In the early stages of evolution of plants, those terpene compounds are predominantly found that are of physiologic importance for the plant: sterols, carotenoids, and the phytol of chlorophyll. To these should perhaps be added the hitherto little-explored sporopollenins, which, as the most resistant of plant materials, form the pollen membrane. Their chemical nature is as yet unknown, but there is reason to believe that a terpenoid with high molecular weight is involved (*69*). Sterols and carotenoids appear early in the course of phylogenetic development. Thus bacteria produce carotenoids and some, such as *Lactobacillus casei*, also sterols (*70*).

Among the algae, the Chlorophyceae (fresh water) form predominantly sitosterol, the Phaeophyceae (mostly warm water) fucosterol, and the Myxophyceae (fresh water) no sterol. Since the last-mentioned class of algae have no sex, Heilbronn believed that there was a relation between sterols and sex (*71*). Of chemotaxonomic interest is the fact that both *Chlorella pyrenoidosa* and *Euglena heliorubrescens* contain ergosterol. This suggests a relationship of the class Euglenaceae to the fungi. Noteworthy is the occurrence of a number of triterpenes in algae: zeorin, leucotylin, ursolic acid, friedelin, *epi*-friedelinol, and taraxerene (*72*). A surprising finding is the occurrence, in green algae, of essential oils with pinene, limonene, cineol, and carvone (*73*).

Among the fungi the production of terpenoid compounds covers a considerably wider range. To be sure, like the bacteria, fungi contain no chlorophyll; neither do they contain the terpenoid phytol. Carotenoids are widely distributed, even though not present in all fungi. The same holds true for the sterols, of which the following have been found to date: ergosterol, zymosterol, dihydro- and dehydroergosterol, cerevisterol, ascosterol, and fungisterol (*69*). In *Penicillium westlingii* the sterol content may be as high as 2.2% of dry weight.

Besides sterols, triterpenes are present in many fungi. Thus eburicoic acid is present in a number of hymenomycetes, up to 20% of dry weight of *Polyporus eucalyptorum* mycelium (*74*). Other triterpenes from fungi include polyporenic acids A, B, and C (*Polyporus* spp. and *Poria cocos*) as well as lanostadienol.

The emergence of terpenes and diterpenes is noteworthy (disregarding the phytol of chlorophyll-containing plants). Thus mycelianamide (CV), which contains an aliphatic terpene residue, was found in



(CV)

TABLE IV

CONSTITUENTS OF SOME *Podocarpus* AND *Dacrydium* SPECIES[a]

Genera and species	Section	Phyllocladene	Rimuene	Ferruginol	g-Ketoferruginol	Totarol	Hydroxytotarol	Podocarpic acid	Conidendrin	Matairesinol	Manool	Manoyl oxide	Ketomanoyl oxide
Podocarpus													
totara	*Eupodocarpus*		+			+	+						
totara var. *hallii*	*Eupodocarpus*	+											
macrophylla	*Eupodocarpus*	+											
ferrugineus	*Stachycarpus*	+		+									
spicatus	*Stachycarpus*	+							+	+			
dacrydioides	*Dacrycarpus*							+					
cupressinum	*Dacrycarpus*							+					
Dacrydium													
cupressinum			+	+	+	+	+	+					
colensoi		+										+	+
biforme		+									+		
bidwillii											+		
kirkii		+									+		

[a] Data from Erdtman (*83*)

TABLE V
Heartwood Constituents of Cupressaceae[a]

Genera and species	Carvacrol	Carvacrol methyl ether	Citronellic acid	α-Thujaplicin	β-Thujaplicin	γ-Thujaplicin	Hydroxy-β-thujaplicin	β-Dolabrin	Nootkatin	Widdrene	Cuparene	Cadinols	Eudesmols	Cedrol	Widdrol	Guaiol	Widdrenic acid	Widdric acid	Other constituents
Callitris																			
calcarata			+													+			
glauca			+													+			Dehydrogeranic acid
intratropica			+													+			
macleayana			+													+			
morrisonii																+			
preissii																+			
propingua																+			
rhomboidalis																+			
roei													+						
verrucosa																+			
Neocallitropsis																			Eudesmenes, dehydrogeranic acid
araucarioides													+						
Widdringtonia																			
cupressoides													+						
dracomontana										+	+		+	+	+		+	+	
juniperoides										+	+			+	+		+	+	Cuparenic acid

schwarzii										+	+			+	+		+	+	
whytei										+	+			+	+		+	+	Cuparenic acid
Tetraclinis																			
articulata	+																		Thymoquinone, hydrothymoquinone
Cupressus																			
bakeri	+								+										
macrocarpa	+	+		+	+	−			+										
macuabiana	+				+				+										
sempervirens	+	+			+				+					+					Cedrene, manool
torulosa	+			+	+		+		+										
Chamaecyparis																			
formosensis				−	−	−	−	−	−										*d*-Myrtenol, *d*-dihydromyrtenol
obtusa				−	−	−	−	−	−										Hinociic acid, hinokiol, hinokione
lawsoniana				−	−	−	−	−	−			+							
nootkatensis	+	+							+										Chamic and chaminic acid
pisifera																			Cadinenes
taiwanensis	+		+																*p*-Cresol, chamenol, hinociic acid
thyoides		+								+	+			+	+		+	+	Cuparenic acid

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[a] From Erdtman (*83*).

TABLE V (*Continued*)

Genera and species	Carvacrol	Hydrothymoquinone	Hydroxythymoquinone monomethyl ethers	Libocedrol	Thujic acid	α-Thujaplicin	β-Thujaplicin	γ-Thujaplicin	Hydroxy-β-thujaplicin	β-Dolabrin	Nootkatin	Cadinenes	"Cedrene"	Widdrene	Cuparene	Cadinols	Cedrol	Ferruginol	Other constituents
Juniperus																			
chinensis	+	+					+	+			+			+	+		+	+?	Hydroxythymoquinone, widdrol, widdrenic acid, widdric acid
communis																		+	Sugiol
excelsa																	+		
mexicana													+				+		
occidentalis						–	–	–	–	–	–						+		
oxycedrus												+				+			
procera													+				+		
virginiana						–	–	–	–	–	–		+				+		
Thuja																			
occidentalis					+	+	+	–											Occidentalol
plicata					+	+	+	+	+	+?									
standishii							+												
Thujopsis																			
dolabrata	+					+	+			+				+					Citronellic acid
Biota																			
orientalis					+	+	+	+						+	+				

Fokienia																			
hodginsi																+			
Pigerhodendron																			
uviferum												+				+			
Heyderia																			
decurrens	+	+	++	+			+	+											Libocedrol, thymoquinone
formosana					+		+												Shonanic acid, chaminic acid
Austrocedrus																			
chilensis	+						+	?			−								

the mycelium of *Penicillium griseofulvum* Dierck (*75, 76*). In the case of mycophenolic acid (from *Penicillium brevi-compactum*), which contains only a fraction of a terpenoid (C_7) (*76*), classification is not possible since the terpenoid fraction could also stem from a carotenoid. The same is true for methylheptenone, a metabolic product of the fungi *Endoconidiophora coerulescens* Münch and *E. rirescens* Davidson. As for diterpene derivatives, rosenonolactone was found (in *Trichothecium roseum* L.) (*76, 77*). In the same fungus, trichothecin was found, presumably derived from a sesquiterpene (*76*). Gibberellic acid, occurring in *Gibberella fujikuroi*, is a diterpene derivative, which commands interest as a growth substance (*76, 78*). In the fern group only one is known (*Paesea scaberula* from New Zealand) in which oxidized sesquiterpenes have been demonstrated with certainty (*79*).

Only the phylogenetically more recent higher plants produce, beyond the physiologically necessary terpenoids—phytol, carotene, and sterols—considerable amounts of mono-, sesqui-, and diterpene compounds. The great mutation necessary for the manufacture of these excretion products could take place only after the plant had attained the means for discarding and taking up the excretion products (*80*). The terpene compounds (essential oils and resins) occurring in large amounts, for example, in conifers could in no instance be identified as storage products. Teleologic explanations for the significance of the terpenes in wound healing and evaporation of water have been refuted. According to present knowledge the terpenes are genuine extracts, i.e., indifferent "ballast" in a prolific metabolic scheme. This does not exclude the possibility that in certain cases terpenes and terpenoids can promote the perpetuation of a species. This holds particularly for sterols and their derivatives (vitamin D, hormones, bile acids, etc.).

Today it is generally conceded that systematically related plants possess similar chemism and thus produce similar materials. The field of investigation concerned with these relationships is designated *chemotaxonomy* (*81*). According to Halliez (*82*), taxonomic studies should obey the rule that simply constructed substances are more widely distributed and systematically less valuable than complex substances formed in long chains of reactions. The latter usually do not exceed the bounds of a genus or family. Fertile fields of chemotaxonomic investigation are to be found in groups such as *Strophanthus, Digitalis, Rauwolfia, Veratrum*, Pinaceae, Agavaceae (saponins), etc. (*81*). Among more recent works, those by Erdtman on heartwood materials of conifers (*83*), by Korte on glycosides (*84*), and by Bathe-Smith on polyphenols (*85*) should be mentioned.

Since terpenes such as limonene, pinene, and the like are found in the green algae as well as in very many higher plants; they have hardly any taxonomic value. More useful in this regard are the rarer terpenoid compounds. Thus, azulogenous guaianolides are typical constituents of the Compositae. For the classification of *Podocarpus* and *Dacrydium* species Erdtman has chosen certain predominant diterpene compounds (Table IV) (*83*); for the classification of the Cupressaceae, he has chosen thujaplicins, mono- and sesquiterpene compounds (Table V) (*83*). It is more difficult to classify the pines on the basis of their turpentine oil composition. Table VI therefore has no outstanding taxonomic value. It does make clear, however, how sharply the turpentines of different pines can be distinguished from one another. Only the compounds longifolene, *n*-heptane, and *n*-undecane can be used for differentiation. Chemotaxonomy of *Eucalyptus* spp. is much easier since rarer terpene compounds can be called upon for comparison. As can be seen in Table VII, there are *Eucalyptus* species that contain predominantly cineol, piperitones, *l*-α-phellandrene, citronellal, geranyl acetate, or *l*-α-pinene (*87*). A similar situation exists for the chemical differentiation of various species of the genus *Orthodon* (Table VIII). Fujita was able to show, through comprehensive investigations, that chemical anal-

TABLE VI

CHEMISTRY OF *Pinus* TURPENTINES (*85–88*)[a]

Pinus species	Origin	*d*-α-Pinene	*l*-α-Pinene	β-Pinene	Δ^3-Carene	*l*-Limonene	*l*-β-Phellandrene	*d*-Cadinene	Longifolene	*n*-Heptane	*n*-Undecane	*l*-Camphene
P. halepensis	Greece	**95**										
P. muricata D. Don	California, Mexico	**99**										+
P. nigra Arnold	Austria		**96**									
P. clausa (Engelmann) Vasey	Florida, Alabama		10	**75**								10
P. ponderosa Laws	California	34		14	**31**	4						
P. pinceana Gordon	Mexico					**80**						
P. contorta Douglas	California, Mexico						**96**					
P. edulis Engelmann	Texas, Mexico	75				5		**20**				
P. longifolia Roxb.	India		24.8	19	37.6				**20.3**			
P. sabiniana Douglas	California									**95**		
P. jeffreyi Murr.	California, Mexico									**95**		
P. coulteri D. Don	California		30–35				34–45			5	**10**	

[a] Numbers are percentages. (Bold face numbers represent predominant constituents.)

TABLE VII

Chemotoxonomy of *Eucalyptus* Species (89)[a]

Eucalyptus species	Cineol	*l*-Phellandral	*l*-Piperitone	*l*-α-Phellandrene	Citronellal	Citronellol	Geraniol	Geranyl acetate	Citral	Eudesmol	Pinene	Terpineol	Aromadendrene	Sesquiterpenes	Camphene	*p*-Cymene	Other constituents
E. globulus	65–75										α +	*dl* +	+		+		*l*-Pinocarveol, *l*-pinocarvone, myrtenol, myrtenal, carvone, verbenone, methyl cinnamate
E. polybractea R. T. Baker	75–90	+														+	Cuminal, *l*-cryptone, australol
E. australiana Baker and Smith	70–75								5		+	α +	?	+			Geranic acid, methyl cinnamate
E. dives Schauer var. C	60–70	+	+	+			+		+	+		α +					Geranic acid, methyl cinnamate
E. dives Schauer		20–30	40–50	+										+	+	+	γ-Terpinene, dipentene, α-thujene, *l*-terpinen-4-ol
E. piperita Sm. type			40–50	+													
E. numerosa Maiden var. A			50	40													
E. dives Schauer var. A			5–15	60–80													
E. australiana var. B	20–50		+	35–40			+			+	+	γ +		+			
E. citriodora Hooker	+				80	25–20	5				+			+			

E. oleosa F. v. M.	45					**20**							+			
E. macarthuri H. Deane and J. H. Maiden							**10**	**60–70**		+	*α* +			+		
E. staigeriana							5	14	**16–40**					+		
E. phlebophylla F. v. M.	10									**20–30**	*l-α* **60**					

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[a] Numbers are percentages. (Bold face numbers represent predominant constituents.)

TABLE VIII
CHEMOTAXONOMY OF *Orthodon* OILS (*90*)[a]

Orthodon species (Family Labiatae)	*d*-Linalool	Thujone	Cineol	Thymol	Carvacrol	Methyleugenol	*l*-Copaene	Dillapiol	Asarone	Ocimene	Orthodene	*d*-Limonene	γ-Terpinene	Pinene	α-Thujene	*d*-Sabinene	Borneol	*p*-Cymene	Carvacrol acetate	Sesquiterpenes	α-Caryophyllene	*l*-Bisabolene	Elemicin
O. linalooliferum Fujita	**80**									+		+								+	+		
O. punctulatum Kudo		**75**										8				11				+	+		
O. lanceolatum Kudo	+		**60–80**								+	+		α +						+	+		
O. leucanthum Kudo				**66**														+					
O. perforatum Ohwi				**56**									+					+		+	+		
O. chinense Kudo	+				**65**										+		+	+	+		+		
O. hadai Kudo		+			**74**													+	+				
O. methylisoeuginoliferum Fujita						**55**	**20**														15	10	
O. formosanum Kudo					9			**62**				*dl*			+			4		+	+	+	2
O. asaroniferum Fujita		+							**25**			+								+	+	+	

[a] Numbers are percentages. (Bold face numbers represent predominant constituents.)

ysis of oils is excellently suited for classification (*88*). The oils of *Ocimum* spp. (oil of basil) also are an example of the possibilities of chemotaxonomic classification. According to the predominance of one or another constituent, ocimum oils can be distinguished from the citral, camphor, thymol, eugenol, and methyl cinnamate types (*89*) (Table IX).

TABLE IX

CHEMOTAXONOMY OF OCIMUM OILS (*91*)[a]

Ocimum types	Citral	Camphor	Thymol	Eugenol	Methyl cinnamate	Ocimene	Myrcene	Geraniol	Geranyl acetate	Citronellol	Citronellal	Citronellic acid	*l*-Linalool	Pinene	Cineol	Terpineol	Carvacrol	Bicyclic sesquiterpenes	Tricyclic sesquiterpenes	Methyl chavicol
Citral type	**68**							26	1.5	+	2.8		+							
Camphor type		**35–77**																		
Thymol type			**40**														+			
Eugenol type				**55–80**		16	+						13	*d*-α +		α +		+		+
Methyl cinnamate type					**55**			0.5					25		1	1		10	4	2

[a] Numbers are percentages. (Bold face numbers represent predominant constituents.)

It may be predicted that chemotaxonomy has great possibilities in the field of botany, as long as those terpene compounds which are used for purposes of comparison are not widely distributed but are specific to certain plant groups. As an example, for the chemotaxonomy of the Leguminosae, diterpene resin acids (vinhaticoic acid, vuocapenic acid, eperuic acid, and cativic acid) might be used as indicators. Triterpenes should hardly be expected to be suited for chemotaxonomic purposes, since they are distributed among the entire plant kingdom (Table X) (*92*).

TABLE X

Occurrence of Triterpenes among the Dicotyledons (*92*)

Classification	α-Amyrin	Uvaol	Brein	Ursolic acid	β-Boswellic acid	Asiatic acid	β-Amyrin	Erythrodiol	Taraxasterol	Morolic acid	Oleanolic acid	β-Amyrin saponin	Lupeol	Betulin	Betulinic acid	Germanicol
Apetales																
Betulaceae									+		+		+	+		
Artocarpaceae													+			
Loranthaceae							+				+		+		+	
Chenopodiaceae											+					
Platanaceae															+	
Dialypetales hypogynes																
Ranunculaceae									+		+					
Lauraceae									+							
Ternstroemiaceae												+				
Euphorbiaceae							+		+		+					
Caryophyllaceae												+				
Linaceae							+	+								
Zygophyllaceae											+					
Rutaceae									+							
Therebinthaceae (Burseraceae)	+		+		+		+					+	+			
Sapindaceae												+				
Polygalaceae												+				
Ilicaceae	+			+												
Celastraceae							+						+			
Rhamnaceae															+	
Ampelidaceae				+							+					
Tiliaceae									+							
Dialypetales perigynes																
Saxifragaceae				+												
Rosaceae				+							+	+		+		
Leguminosae	+						+			+	+	+	+			
Umbelliferae						+										
Araliaceae												+				
Cornaceae				+											+	
Myrtaceae				+							+				+	
Cactaceae								+			+					
Gamopetales hypogynes																
Ericaceae		+		+			+			+	+					
Primulaceae												+				
Sapotaceae	+						+						+			
Styraceae												+				
Oleaceae				+							+					

TABLE X (*Continued*)

Classification	α-Amyrin	Uvaol	Brein	Ursolic acid	β-Boswellic acid	Asiatic acid	β-Amyrin	Erythrodiol	Taraxasterol	Morolic acid	Oleanolic acid	β-Amyrin saponin	Lupeol	Betulin	Betulinic acid	Germanicol
Loganiaceae	+															
Apocynaceae	+			+			+				+		+		+	
Asclepiadaceae				+			+		+							
Gentianaceae											+				+	
Solanaceae				+							+					
Scrophulariaceae												+			+	
Verbenaceae				+												
Labiatae				+							+					
Plantaginaceae				+							+					
Cucurbitaceae												+				
Rubiaceae											+					
Caprifoliaceae															+	
Compositae							+		+		+					+

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CHAPTER 12

Quinones: Structure and Distribution

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I. Introduction

The quinones form a large group of natural coloring matters with representatives in several phyla, the majority being found in flowering plants and fungi. They are more numerous, more varied in structure, and more widely distributed (carotenoids and melanins excepted) than other classes of natural pigments. The major sources are listed in Table I; a few others, found in different phyla, are discussed later. Little systematic work has been done on the natural distribution of quinones, and it is likely that further search will extend the range of these pig-

TABLE I
NATURAL DISTRIBUTION OF QUINONES

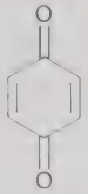
Source	Approximate number of quinones
Plant kingdom	
Flowering plants	80
Fungi	55
Lichens	10
Bacteria	15
Animal kingdom	
Arthropoda (mainly insects)	15
Echinodermata (mainly sea urchins)	15

ments. In contrast to the carotenoids, individual quinones are seldom widely dispersed, and, until recently, the occurrence of the same quinone in both the animal and plant kingdoms was unknown, although closely related quinones are found in both. The exceptions to this are the ubiquinones or coenzymes Q—a family of quinones that appears to be very widely distributed, notably in mammals, including man—and possibly also plastoquinone and vitamin K_1, which may exist generally in green leaves. It must be emphasized however, that there are large gaps in our knowledge of the distribution of quinones, so that, as yet, structure can be correlated with taxonomy only to a limited extent. Fortunately this situation is steadily improving as the isolation of new quinones from natural sources continues.

The chemistry of the naturally occurring quinones is well established (*1*); there are comparatively few whose structures are not known completely, and the great majority have been synthesized in the laboratory. In contrast, their biochemistry is not well understood. Considerable progress has been made in the study of their biogenesis, but otherwise we have little knowledge of their metabolism or function, with the exception again of the coenzymes Q and vitamins K, which have recently been subjected to intensive investigation. In many cases the quinone pigments appear to be merely metabolic by-products. Occasionally they are formed in surprisingly large amounts, notably by fungi, yet mutants may thrive when the production of quinones is completely blocked. Much quinone biogenesis is probably a deviation from fatty acid metabolism, a metabolic shunt which comes into play when the enzyme systems become saturated with respect to their substrates. As many of the pigments so formed have antibiotic properties, their existence is no doubt advantageous to the organisms that produce them.

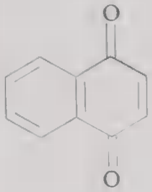
In general, the quinones are yellow to red, or brown, in color, but where present as salts (of, e.g., hydroxyquinones) the range may extend to purple, blue, or green. (Black polymeric quinones are not considered in this chapter.) Regarded simply as coloring matters they are of little importance; many occur in bark or roots or are formed by microorganisms, and only those few present in the higher fungi and lichens or in the spines and tests of sea urchins, make a significant contribution to natural coloring. Occasionally a quinone and the corresponding quinol occur together; sometimes only the colorless quinol has been found.

The naturally occurring quinones can be divided chemically into the following groups: benzoquinones, 40; naphthoquinones, 40; anthraquinones, 70; and miscellaneous quinones, 25, which include tetracenequinones, phenanthrenequinones, and extended quinones. [In extended quinones the two carbonyl groups are located in different rings, e.g.



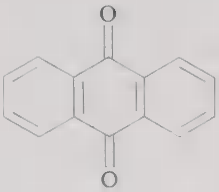
Benzoquinone

(I)



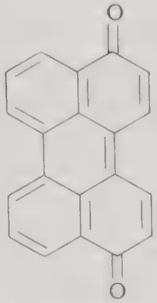
Naphthoquinone

(II)



Anthraquinone

(III)



Perylenequinone

(IV)

(IV).] Dibenzoquinones, dianthraquinones, and one dinaphthoquinone are also known. There is considerable variation in the pattern and structure of substituents, and the diversity of molecular architecture may be illustrated by ubiquinone$_{50}$, a benzoquinone possessing a side chain of fifty carbon atoms, the hexahydroxynaphthoquinone spinochrome E, and the aphin pigments, which are coronene derivatives. Only the parent *p*-quinones are illustrated (I, II, III), as *o*-quinones are of rare occurrence, and *o*-benzoquinones are known only as intermediate metabolites which have never been isolated.[*] This is not surprising as they are extremely reactive compounds and would not be expected to survive for long *in vivo*. However, reactive compounds have been found in nature before, and in view of the numerous catechol derivatives that exist the future isolation of a naturally occurring *o*-benzoquinone cannot be excluded, especially if it be heavily substituted. [With the exception of diosquinone (cf. XLIV, below), all the natural *o*-quinones discovered so far have a fully substituted quinonoid ring.]

It is not intended to discuss the chemistry of quinones in this chapter, but certain aspects should be borne in mind when considering the possible functions of these compounds. Only the characteristic quinone addition reactions are likely to be of consequence *in vivo*, and in particular the so-called 1,4-type of addition. Three modes of addition are possible for simple quinones, namely, addition to the structures

$$(a)\ \overset{1}{\mathrm{C}}{=}\overset{2}{\mathrm{C}},\quad (b)\ \overset{1}{\mathrm{C}}{=}\overset{2}{\mathrm{C}}{-}\overset{3}{\mathrm{C}}{=}\overset{4}{\mathrm{O}}\ \text{and}\ (c)\ \overset{1}{\mathrm{O}}{=}\overset{2}{\mathrm{C}}{-}\overset{3}{\mathrm{C}}{=}\overset{4}{\mathrm{C}}{-}\overset{5}{\mathrm{C}}{=}\overset{6}{\mathrm{O}}$$

1,2-Additions to the activated ethylenic double bond (*a*), typically Diels-Alder reactions, addition of chlorine, formation of epoxides, etc., are probably of little consequence in this context; but 1,4-additions to the α,β-unsaturated ketone system (*b*), especially addition of thiols and primary or secondary amines, are of great importance in the formation of certain complex polyquinonoid structures and may be significant in the biological functioning of a few simple quinones. As shown (Reaction 1) the initial addition reaction is followed by enolization of the remaining carbonyl group to form a substituted quinol, which can then be oxidized to the final product by the original quinone, since introduction of amino or thiol groups lowers the redox potential. Benzoquinone itself is very reactive, and with amines it is difficult to stop the reaction at the monoaminoquinone stage; the major product is usually a 2,5-diaminoquinone. Thiols are more reactive than amines, and an extreme case is the interaction of equimolecular amounts of benzoquinone and thioglycolic acid at room temperature, which leads to the rapid separation of the tetra-substituted quinol (V) (2). There is some evidence (3) that the antibiotic activity of certain quinones arises from their ability to react in this way with essential thiol groups in the bacterial cell. This could be true of simple alkylated benzoquinones, e.g., gonyleptidine (p. 679). It is important to remember that 1,4-addition reactions can take place only with quinones having an unsubstituted position in the quinone ring, i.e., *ortho* to carbonyl. (A methoxyl group attached to a quinone ring could be replaced by reaction with an amine or thiol, but very few natural quinones of this type are known.) All the anthraquinones and more complex quinones are thus excluded, and also a majority of the natural naphthoquinones and benzoquinones, which are either fully substituted in the quinone ring or would be unlikely to react by 1,4-addition for other reasons. This leaves a mere handful of simple quinones whose ability to react in this manner might be connected with their biological function. It is possibly significant that these few reactive compounds include a group of alkylated benzoquinones secreted

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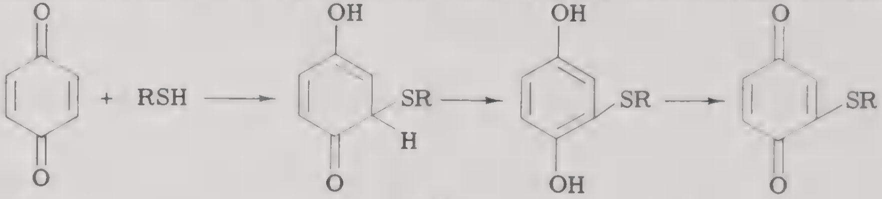
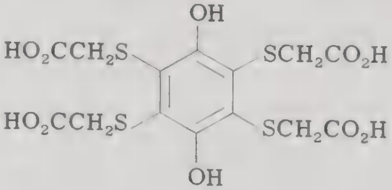
[*] The occurrence of quinones as intermediate metabolites is not considered in this chapter.



(1)



(V)

by certain insects as a means of defense, and one or two simple naphthoquinones (e.g., juglone, plumbagin) known to be poisonous to animals and plants. However, toxicity is not restricted to simple structures, for a number of more complex quinones (e.g., skyrin) occurring in plant pathogens are harmful to various organisms.

The only example of 1,6-addition to the conjugated system (*c*, above) to be considered is that of hydrogen. Reversible reduction is virtually the only reaction common to all the naturally occurring quinones. It has long been thought that quinones might participate in cellular respiration, and the coexistence of a quinone and its corresponding quinol, e.g., in cultures of *Aspergillus fumigatus* Fresenius, supports this view. Although this may be true in a few instances, it is probably not the case in general. However, it has recently been established that coenzyme Q (ubiquinone) is an active member of the respiratory chain in mammalian mitochondria, and there is now evidence that plastoquinone plays a vital part in photosynthetic phosphorylation. The new chloroplast pigment plastoquinone may also be involved in electron transport in photosynthesis. These important functions, dependent upon the property of reversible oxidation-reduction, are probably confined to a comparatively few, widely distributed, lipid-soluble, quinones. In all other cases, definite evidence concerning the function, if any, of natural quinones is lacking, and in view of the considerable variations both in their structure and properties, and in their location within living tissues, it is unwise to make generalizations about their behavior.

II. Distribution of Quinones in Nature

A. THE PLANT KINGDOM

1. *Flowering Plants*

a. Angiospermae. The largest group of natural quinones occurs in flowering plants. Nearly half of them are found in one family, the Rubiaceae, and the rest are dispersed among some thirty to forty families only, so that our present knowledge of the distribution of quinones in angiosperms is very limited.* The few systematic surveys of plant quinones which have been recorded are all concerned with anthraquinones, and the information available comes largely from investigations on plants of economic, or potentially economic importance, mainly those used as dyes or drugs. As a result the anthraquinones now have some taxonomic value, but this is hardly the case, as yet, with the others, and caution is needed in deducing phylogenetic relationships from the presence of the same, or similar, quinones in different plants. Anthraquinones for example, occur in the monocotyledonous Liliaceae as well as in several dicotyledonous families, and there are examples in the benzoquinone and naphthoquinone series of similar quinones appear ing in apparently unrelated families. Thus, 2,6-dimethoxybenzoquinone is found in the Simarubeae and the Meliaceae, which occupy similar systematic positions, but they have little connection with the Ranunculaceae, another source of the same quinone. Hydroxynaphthoquinones of similar structure occur in the Droseraceae, Plumbaginaceae, and Juglandaceae; again the first two families are related, but not the third. The existence of the same or very similar quinones in quite unrelated plants suggests that they originate from very simple precursors and may be of little phytochemical significance; it must also be borne in mind that unrelated plants may produce the same substance by different biochemical pathways.

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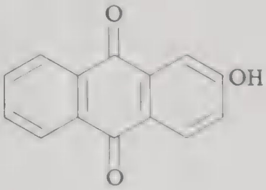
* Only quinones that have been isolated and identified are considered. No attention is paid to the numerous claims to have detected quinones, based solely on color reactions, as these are not always reliable and in any case do not appreciably increase the number of quinone-forming families.

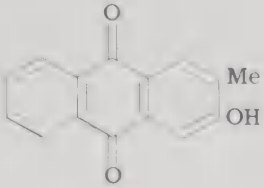
(*1*). *Anthraquinones.* The distribution of anthraquinones in flowering plants has been reviewed recently by Hegnauer (*4*). In practice, they have usually been isolated from roots and bark, although the pigments are not confined to these tissues as several histochemical studies have shown (*5*). In structure the anthraquinones can be divided roughly into two groups: those that occur in the Rubiaceae (about 30 quinones) and those which do not (about 10 quinones). The latter are found mainly in the Polygonaceae (particularly *Rumex* and *Rheum* spp.), Rhamnaceae, and Leguminosae (mainly *Cassia* spp.). Members of both groups frequently occur as glycosides, a feature not encountered in the natural benzoquinones or naphthoquinones. The majority are polyhydroxy derivatives of 2-methylanthraquinone but the pattern of substitution differs in the two groups, which may be a consequence of their different biogenetic origins. The β-methyl group occurs in various states of oxidation, $—CH_3$, $—CH_2OH$, $—CHO$, $—CO_2H$, or it may be absent altogether, but no other side chain is found in the angiospermous anthraquinones in contrast to many of those produced by lower plants and animals.

The structures of the rubiaceous quinones (VI–XXIII) and most of the other anthraquinones (XXIV to XXXIII) are shown; Table II gives the distribution of the second group. There are two anthraquinones that lack hydroxyl groups: anthraquinone itself, found in quebracho tannin extracts (*Quebrachia lorentzii* Engl.) (*6*) and possibly also in wattle extracts (*Acacia decurrens* Willd) (*6*), and 2-methylanthraquinone

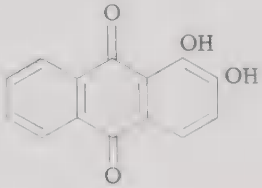
Anthraquinones in the Rubiaceae[a]



(VI)

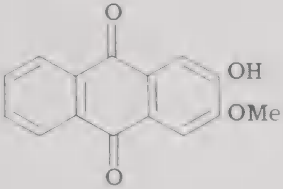


(VII)



Alizarin

(VIII)



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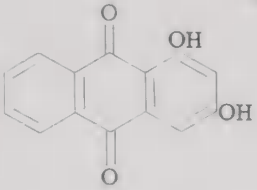
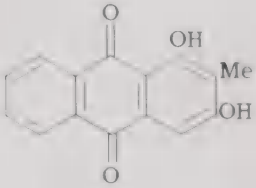


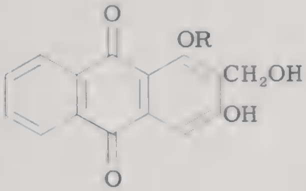
Xanthopurpurin

(X)



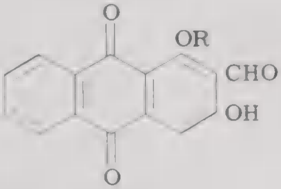
Rubiadin

(XI)



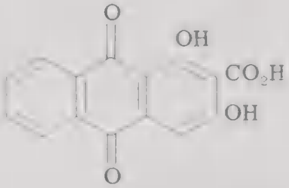
Lucidin (R=H)
Damnacanthol (R=Me)

(XII)



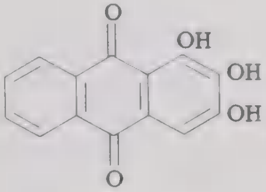
Damnacanthal (R=Me)
Nordamnacanthal (R=H) [b]

(XIII)



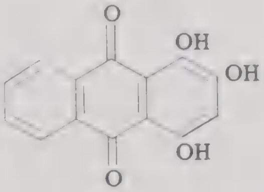
Munjistin

(XIV)



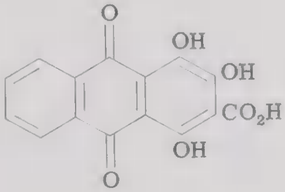
Anthragallol

(XV)



Purpurin

(XVI)

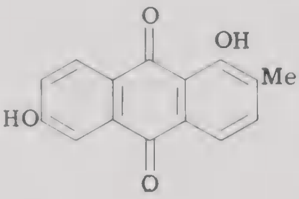


Pseudopurpurin

(XVII)

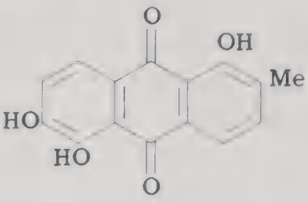
(*continued*)

Anthraquinones in the Rubiaceae[a] (continued)



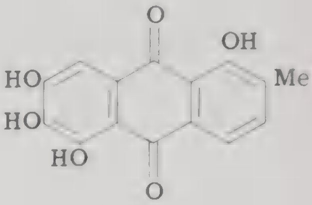
Soranjidiol

(XVIII)



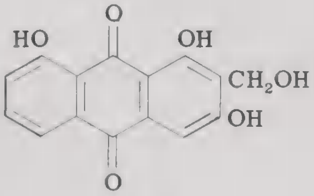
Morindone

(XIX)



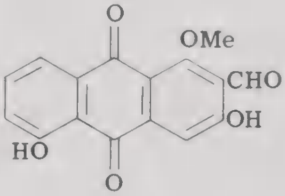
Copareolatin

(XX)



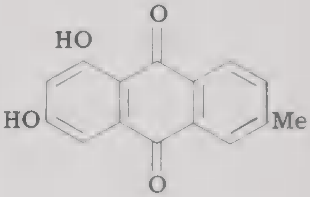
Coelulatin[c]

(XXI)



Juzunal[d]

(XXII)



(XXIII)

[a] For references to individual species see (*1*) except where indicated. Methyl ethers of parent hydroxyquinones are mentioned only when they have different trivial names.

[b] From the heartwood of *Morinda tinctoria* Roxb. (*26a*).

[c] Occurs in *Coelospermum reticulatum* Benth. (*9*).

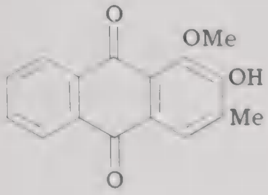
[d] Structure now established (*10*).

(tectoquinone) present in teak heartwood (*Tectona grandis* Linn.)[*] (*7*). It will be seen that in most of the rubiaceous quinones the hydroxyl groups are confined to one ring, whereas in the second group, of which emodin (XXV) is typical, hydroxyl groups are nearly always present in both benzenoid rings. 6-Methylxanthopurpurin (XXIII) is structurally in the emodin group, although isolated from a rubiaceous plant (*Morinda umbellata* Linn.) (*8*); unfortunately the constitution of this anomalous quinone has not been definitely established. Coelulatin (XXI), a recent addition to the rubiaceous quinones, is exceptional in possessing hydroxyl groups in the 1,8-positions hitherto regarded as a characteristic feature of the emodin type of pigment.

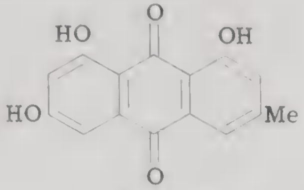
Chrysophanol (XXVI) occurs in several plant families, frequently

[*] Together with 2-hydroxymethylanthraquinone, anthraquinone-2-carboxylic acid, and possibly the 2-aldehyde (*7a*).

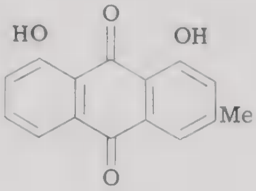
Anthraquinones not found in the Rubiaceae


Digitolutein

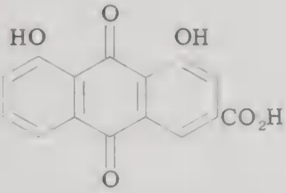
(XXIV)


Emodin
(Physcion is the 6-Me ether)

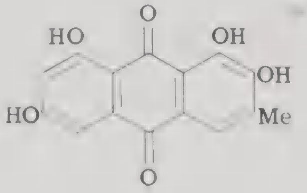
(XXV)


Chrysophanol

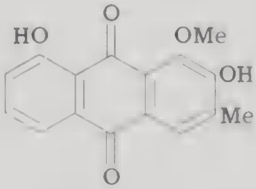
(XXVI)


Rhein

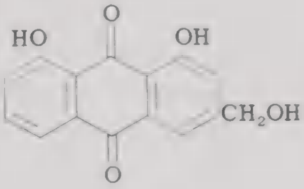
(XXVII)


Alaternin[e]

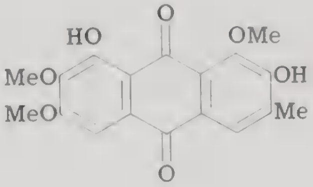
(XXVIII)


Obtusifolin[f]

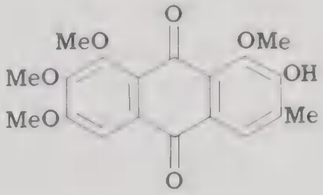
(XXIX)


Aloe-emodin

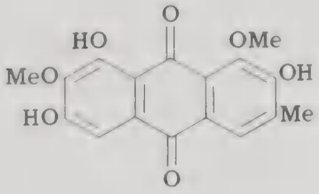
(XXX)


Obtusin[f]

(XXXI)


Chryso-obtusin[f]

(XXXII)


Aurantio-obtusin[f]

(XXXIII)

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[e] Structure now established by synthesis (*26*).

[f] From the seeds of *Cassia obtusifolia* Linn. (Leguminosae) (27).

together with emodin, and both of these, and/or related quinones, are known in fungi and lichens. In contrast, none of the quinones produced by the Rubiaceae have been found elsewhere with the exception of 3-hydroxy-2-methylanthraquinone (VII) which is present in teak wood (Verbenaceae) (*11*) along with 2-methylanthraquinone. Quinones of the emodin group are sometimes accompanied by the corresponding anthrones, both in flowering plants and in fungi, and occasionally only the anthrone is found; e.g., the 9-anthrone of chrysophanol occurs alone in the heartwood of *Ferriera spectabilis* Allem (Leguminosae) (*12*), and both anthrones of physcion (emodin 6-methyl ether) have been found in the root bark of *Ventilago maderaspatana* (Gaertn.) (Rhamnaceae) (*13*). The presence of anthrones is of biogenetic significance, and it is noteworthy that anthrones have not been found in the Rubiaceae. The anthrone barbaloin (XXXIV) is unusual in two respects; it is a *C*-glucoside (*14*)* and it occurs in monocotyledonous plants of the Liliaceae family.† [Chrysophanol has been found, as an artifact, in resins obtained from related *Xanthorrhoea* spp. (*14b*).] On oxidation it yields aloe-emodinanthrone and aloe-emodin, both of which are usually

TABLE II

DISTRIBUTION OF ANTHRAQUINONES (EXCLUDING THE RUBIACEAE)

Quinone	Family
Anthraquinone	Anacardiaceae
2-Methylanthraquinone	Verbenaceae
3-Hydroxy-2-methylanthraquinone (VII)	Verbenaceae
Digitolutein (XXIV)	Scrophulariaceae
Emodin (XXV)	Leguminosae, Lythraceae, Polygonaceae, Rhamnaceae
Physcion (XXV)	Leguminosae, Polygonaceae, Rhamnaceae
Chrysophanol (XXVI)	Ericaceae, Euphorbiaceae, Leguminosae, Lythraceae, Polygonaceae, Rhamnaceae, Saxifragaceae
Rhein (XXVII)	Leguminosae, Polygonaceae
Alaternin (XXVIII)	Rhamnaceae
Obtusifolin (XXIX)	Leguminosae
Obtusin (XXXI)	Leguminosae
Chryso-obtusin (XXXII)	Leguminosae
Aurantio-obtusin (XXXIII)	Leguminosae
Aloe-emodin (XXX)	Leguminosae, Liliaceae, Polygonaceae, Rhamnaceae

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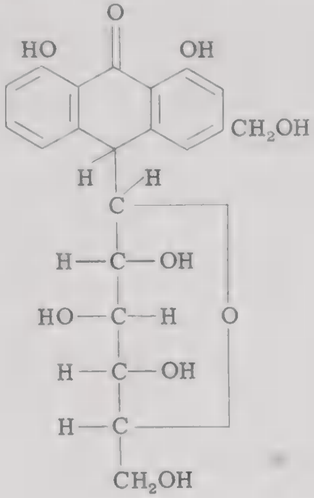
[*] Homonataloin (from Natal aloes) is another example of the same type of structure (*14a*).

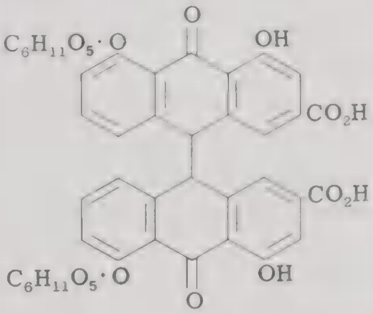
[†] For references to the occurrence of anthraquinones in Liliaceae see (*4*).



Barbaloin

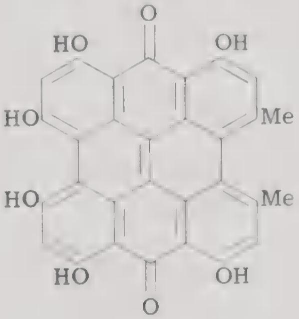
(XXXIV)

present in aloes, an exudate from the leaves of several *Aloe* spp., which is used as a purgative. Many natural laxatives (rhubarb, cascara, senna) contain mixtures of anthraquinones and anthrones of the emodin type, both as glycosides and in the free state, and dianthrones may also be present. The active principles of senna pods and leaves (*Cassia angustifolia* Vahl, Leguminosae) are two stereoisomeric diglucosides of dirhein-9-anthrone (XXXV), known as sennosides A and B (*15*). A similar glycosidic diemodinanthrone is present in the bark of the alder buckthorn (*Rhamnus frangula Linn.*) (*16*). A more complex extended quinone, closely related to emodin, is the photodynamic pigment hypericin (XXXVI), present in the petals, stems, and leaves of many Hypericaceae (*17*). This family has no obvious botanical relationship



Sennosides A and B

(XXXV)



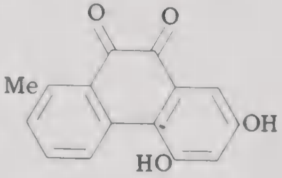
Hypericin

(XXXVI)

with any of the foregoing emodin-producing plants, but fagopyrin (*17*), which is a derivative of hypericin, occurs in the flowers of buckwheat (*Fagopyrum esculentum* Moench), a member of the Polygonaceae.

As noted above, emodin-type athraquinones frequently occur as mixtures of related compounds, and this is usually the case in the Rubiaceae. Small amounts of unidentified quinones are often isolated in addition to the principal coloring matters (*18*). From the roots of the madder plant (*Rubia tinctorum* Linn.), formerly a very important natural dyestuff, the following quinones have been isolated: alizarin (VIII), xanthopurpurin (X), rubiadin (XI), munjistin (XIV), purpurin (XVI), and pseudopurpurin (XVII). The bark of the New Zealand shrub *Coprosma acerosa* A. Cunn, used by the Maoris for dyeing purposes, contains 3-hydroxy-2-methylanthraquinone (VII), rubiadin methyl ether (XI), lucidin (XII), soranjidiol (XVIII), anthragallol 2-methyl ether and 1,2-dimethyl ether (XV). Occasionally these pigments occur in substantial amounts, as in the bark of *Coprosma australis* Robinson, of which morindone (XIX) and its glycoside morindin, together with rubiadin 1-methyl ether constitute 17% of the dry weight, besides minor constituents (*19*). On the other hand, there are several records of the solitary occurrence of an anthraquinone in various plants unaccompanied by other anthracene compounds, e.g., chrysophanol in *Saxifraga delavayi* Franch (Saxifragaceae) (*20*) and alizarin in *Hedyotis auricularia* (Rubiaceae) (*21*); digitolutein (XXIV) is the lone anthraquinone in the leaves of two *Digitalis* spp. (Scrophulariaceae) (*22*).

(*2*). *Phenanthrenequinones.* The only phenanthrenequinone comparable to the foregoing anthraquinones is the pigment denticulatol



Denticulatol

(XXXVII)

found in the root of *Rumex chinensis* Campd. (Polygonaceae) (*1*, *23*), which is used occasionally in China as a substitute for rhubarb. It is isomeric with chrysophanol, a common *Rumex* constituent also present in *R. chinensis* and is possibly (XXXVII).* The tanshinones (*1*, *24*) are orange to red pigments found in the root of *Salvia miltiorrhiza* Bge.

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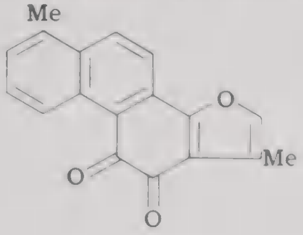
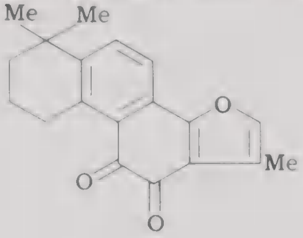
* The isomeric structures proposed by Chi *et al.* (23) have been disproved by synthesis (*23a*).

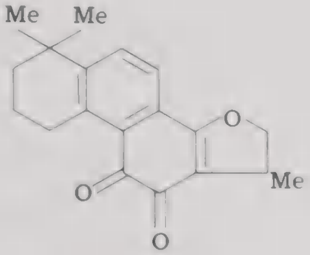
(Labiatae), another Chinese purgative drug. The most probable structures, shown below, bear a close relationship to abietic acid. Terpenes are abundant in the Labiatae, and these are essentially terpenoid pigments.



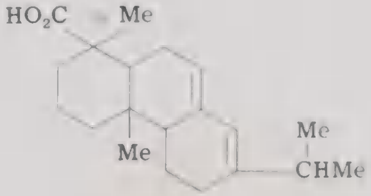
Tanshinone I



Tanshinone II



Cryptotanshinone

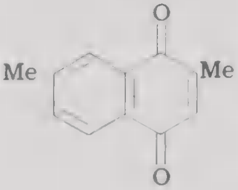


Abietic acid

(3). *Naphthoquinones.* All the naphthoquinones of known structure which have been found in flowering plants are shown (XXXVIII–LIV). The distribution of individual compounds is limited, few quinones occurring in more than one family of plants. The existence of plumbagin (XLVIII) in three related families, and of lapachol (XLI) in the Bignoniaceae and Verbenaceae, is consistent with the botanical classification of these plants, but the appearance of lawsone (XXXIX) in both Lythraceae and Balsaminaceae is unexpected. Although naphthoquinones and anthraquinones may exist in the same family, they usually show little structural resemblance (cf. cordeauxiaquinone (LIII), vitamin K_1 (LIV), and aloe-emodin (XXX), in the Leguminosae), but in the one instance where a quinone from each class (lapachol and tectoquinone) occurs in the same species (*Tectona grandis*) (25) they are obviously related (see p. 712). As in the anthraquinone group, there is one naphthoquinone, eleutherin (LI) (more precisely two stereoisomers), among the monocotyledons; it occurs in the tubers of *Eleutherine bulbosa* (Mill.) Urb. (28), and shows some structural resemblance to the fungal pigments javanicin and fusarubin.

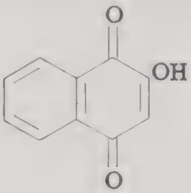
The quinones (XLVI), (XLVIII), (XLIX), and (L) occur in re-

Structure and Distribution of Naphthoquinones in Flowering Plants[a]



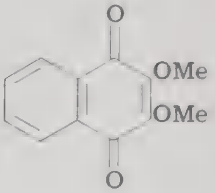
Chimaphilin
(Ericaceae)

(XXXVIII)



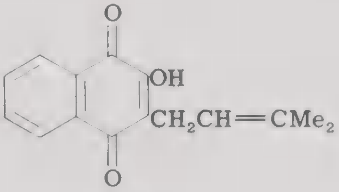
Lawsone
(Lythraceae,
Balsaminaceae)(*47*)

(XXXIX)

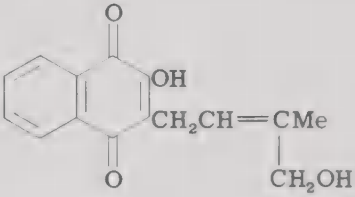


(Balsaminaceae)

(XL)



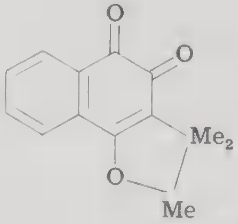
Lapachol
(Bignoniaceae,
Sapotaceae,
Verbenaceae)
(XLI)



Lomatiol

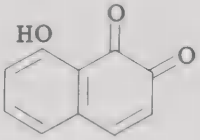
(Proteaceae)

(XLII)



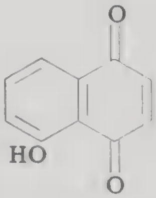
Dunnione
(Gesneraceae)

(XLIII)



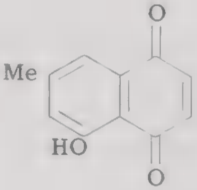
Diosquinone
(Ebenaceae)

(XLIV)



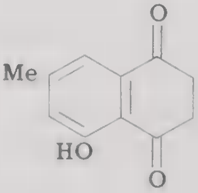
Juglone
(Juglandaceae)

(XLV)



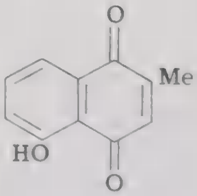
(Ebenaceae)

(XLVI)

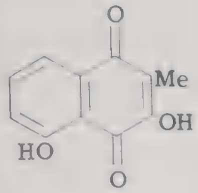


(Ebenaceae)

(XLVII)

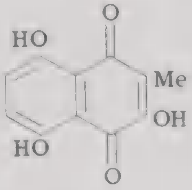


Plumbagin
(Plumbaginaceae,
Droseraceae,
Ebenaceae)
(XLVIII)



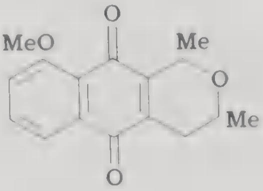
Droserone
(Droseraceae)

(XLIX)



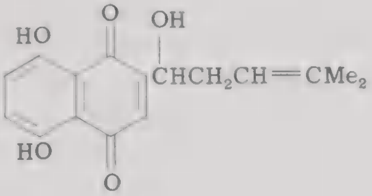
Hydroxydroserone
(Droseraceae)

(L)



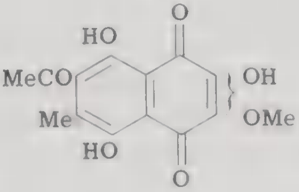
Eleutherin[b]
(Iridaceae)

(LI)



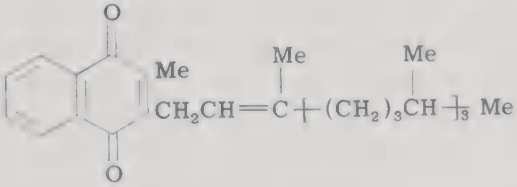
Alkannin[c]
(Boraginaceae)

(LII)



Cordeauxiaquinone
(Leguminosae)

(LIII)



Vitamin K
(Leguminosae)

(LIV)

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[a] For references to individual species see (*1*) except where indicated.

[b] An optical isomer, isoeleutherin, occurs in the same plant.

[c] An optical isomer, shikonin, also occurs naturally (*48*).

lated families and have similar structures reminiscent of the emodin-chrysophanol type of anthraquinone. Droserone is present with hydroxydroserone in the pseudo-bulbs of *Drosera whittakerii* Planch (*29*), and with plumbagin in the tuberous roots and rhizomes of *D. peltata* Sm. (*30*). [A yellow pigment isolated along with plumbagin from *D. rotundifolia* Linn. is a flavone and not a naphthaquinone-carboxylic acid as has been suggested (*31*)]. Plumbagin (2-methyljuglone) (XLVIII) is also found with 7-methyljuglone (XLVI) in the bark, leaves, and fruit of *Diospyros ebenum* Koen (*32*). A third compound found in the fresh leaves and immature fruits of this shrub is the diketone (XLVII), the only example of the natural occurrence of such a "β-hydronaphthoquinone" (*32*). Another pigment found in the *Diospyros* genus is the orange-red *o*-quinone, diosquinone (XLIV) isolated from the bark of *D. tricolor Hiern* (*33*). An orange isomer, juglone (XLV), is similar in structure to plumbagin and related quinones but confined apparently to members of the Juglandaceae (*1*, *34*). It exists in a reduced form, as the 4-β-D-glucoside of α-hydrojuglone (1,4,5-trihydroxynaphthalene) (*35*, *36*), in most parts of the walnut tree, subject to seasonal variation (*37*). The highest concentration (<8%) occurs in male catkin buds in the dormant winter state, but the quinone is most conveniently obtained from green walnut shells. A phenoloxydase has been isolated from the husks, and when this is added to an aqueous solution of hydrojuglone glucoside, juglone soon appears (*35*). It has often been observed that walnut trees are poisonous to surrounding plants, which sometimes creates problems in horticulture (*38*). Juglone is generally considered to be the toxic principle, and the experiments of Bode (*39*) indicate that it is carried down from the leaves onto neighboring plants by rain. Walnut shells and the fruits of various *Diospyros* species have been used to poison fish, *D. ebenum* has been suspected of poisoning livestock in Australia, and the sap of *D. maritima* Blume is vesicant. All these plants contain juglone or *C*-methyljuglones, either as such or in a reduced form.

The only other chemically related group of naphthoquinones consists of lapachol (XLI), lomatiol (XLII), and the *o*-quinone dunnione (XLIII); in the latter, which is isomeric with lapachol, the isopentenyl side chain has become part of a five-membered ring. [There is a third *o*-naphthoquinone, biflorin, which occurs in the leaves and petals of *Capraria biflora* Linn. (Scrophulariaceae) (*40*). Its structure has not yet been elucidated.] These three quinones have been isolated from different parts of quite unrelated plants. The yellow pigment lapachol occurs principally in the heartwoods of the Bignoniaceae [7.6% in *Tecoma araliacea* DC. (*41*)], whereas dunnione is found as an orange-red deposit on the leaves, stems, and flowers of *Streptocarpus Dunnii* Mast., the very large leaves bearing as much as 2 gm. of pigment (*42*). Lomatiol forms the yellow outer layer of the seeds of a number of (but not all) *Lomatia* spp. (*43*). In *L. longifolia* R.Br. it is found also in the ripe fruits (3.5%) but more abundantly in the seeds (12%), and it may be present in the bark of *L. ilicifolia*, which has been used for dyeing purposes. Several naphthoquinone-containing plants have been used as natural dyes, but none ever attained the importance of the anthraquinones. Lawsone (XXXIX) is the dyeing principle of henna, obtained from the leaves of *Lawsonia alba* Lam. (Lythraceae) (*44*), and the root of Dyer's bugloss (*Alkanna tinctoria* Tausch.) (Boraginaceae) (*45*) contains alkannin (LII) in the form of its angelic ester. *Onosma echioides* Linn. (Boraginaceae) is said to contain 19.4% of alkannin in the root bark (*46*).

Vitamin K_1. The best known naphthoquinone produced by higher plants is vitamin K_1, 2-methyl-3-phytyl-1,4-naphthoquinone (LIV), which was isolated from alfalfa leaves (*Medicago sativa* Linn.) as a light-yellow oil by Dam and Karrer and their colleagues (*49*) and by Doisy and co-workers (*50*), who determined its structure. Several syntheses have been described (*51*). After his recognition that the hemorrhagic condition produced in chicks kept on an ether-extracted diet was due to a new fat-soluble factor, Dam, and others, surveyed many possible sources of this new vitamin (*51*). They found that an antihemorrhagic factor was widely distributed in green plants: chestnut leaves, cabbage, spinach, and nettles proved to be rich sources, but the active principle was not identified. (Plastoquinone and ubiquinone are probably present in all the plants that were examined, but these quinones do not have vitamin K activity.) The findings of Dam, and others, were based on bioassay procedures that do not distinguish between 2-methyl-3-phytyl-1,4-naphthoquinone and other active compounds, and it is unfortunate that these bioassays have not been followed by chemical investigation, except in the case of alfalfa. From many compounds tested it has been demonstrated that vitamin K activity is limited to 2-methyl-1,4-naphthoquinones (or naphthalene precursors easily oxidized thereto) unsubstituted in the benzene ring and alkylated at position 3, a C_{20} side chain giving optimal activity (*52*). Hence the active compounds occurring in green leaves may comprise a family of isoprenologs related to 2-methyl-3-phytyl-1,4-naphthoquinone, including perhaps 2-methyl-1,4-naphthoquinone itself.

Both vitamin K_1 and chlorophyll possess a phytyl side chain, and some early experiments (*53*) with germinating seedlings suggested that the biosynthesis of vitamin K_1 ran parallel with that of chlorophyll. The apparent localization of vitamin K* in chloroplasts led Dam (*54*) to suggest, about twenty years ago, that it might be involved in photosynthesis, and a tentative mechanism was proposed later by Wessels (*55*). Bishop (*56*) has found recently that the photochemical activity of isolated chloroplasts can be reversibly inhibited by extraction with light petroleum. Although the activity of extracted chloroplasts can be

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[*] Hereinafter the term vitamin K refers to an unidentified compound(s) having vitamin K activity which may or may not be identical with one of the known K vitamins.

restored by addition of vitamin K_1, the light petroleum extract does not contain vitamin K, but rather an appreciable amount of plastoquinone. [Other investigators (*56*) confirm that vitamin K is absent from chloroplasts. Dam's original work was evidently done on somewhat crudely separated fractions.] It is generally accepted that a quinone participates in photosynthetic electron transport (most probably in photosynthetic phosphorylation), but the precise sequence of events is still obscure. Various schemes have been proposed (see Vol. V of this treatise and ref. *57*) which should now be modified by substituting plastoquinone for vitamin K. [The function of vitamin K is discussed further on p. 696.]

(*4*). *Benzoquinones.* All aromatic compounds of the anthracene series found in plants are quinones, and most of the naphthalene compounds which have been isolated are also quinones. In contrast, few benzoquinones have been discovered although benzenoid compounds, including phenols, are common plant products. Of the eight quinones shown on pp. 650–651, perezone (LXI), found in roots of two Compositae (*58*), is a sesquiterpene, and the pedicinins (LXVI) are two of a group of closely related polyhydroxychalcones and flavanones present as a yellow dust on the leaves of *Didymocarpus pedicellata* R.Br. (*59*). A major component of this mixture is a chalcone-quinol, pedicin, from which the pedicinins are derived, but the pigments as a group are probably best regarded as chalcones, two of which happen also to be quinonoid.* Thymoquinone has been isolated, along with thymoquinol (LVIII) from the essential oil of *Monarda fistulosa* Linn. (Labiatae) (*60*) but is probably an artifact. The three orange pigments (LXIII), (LXIV), and (LXV), occur chiefly in the berries of mysinaceous shrubs, but embelin is present also in the root of *Embelia barbeyana* Mez. (*61*) and rapanone has been found both in the bark of *Rapanea maximowiczii* Koidz (Myrsinaceae) (*62*) and in the bulbs of *Oxalis purpurata* var. *jacquinii* (Sonder) (Geraniaceae) (*63*). Structurally related phenols with long alkyl side chains also occur in higher plants, not in the Myrsinaceae but in several genera of the Anacardiaceae. In particular, the oily exudate from *Campnosperma auriculata* Hook.f. contains a mixture of alkylated quinols of which nonadecylquinol (LXII) has been identified (*64*). (Maesaquinone has also a C_{19} side chain.) Mixtures of catechols with a C_{15} side chain at position 3 occur in the oil exuded by *Rhus toxicodendron* Linn. (*65*), *Semecarpus anacardium* Linn. (*66*), and other species, whereas those obtained *inter*

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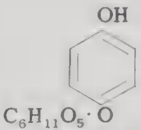
* There is a similar group of coloring matters in the flowers of *Carthamus tinctorius* Linn. (Compositae) which includes the chalcone-quinone carthamone (*59a*).

alia from *R. ambigua* Lav. (*67*) and *S. vernicifera* Hayata (*67*) have a C_{17} side chain. Thitsiol is a catechol alkylated (C_{17}) at position 4 (*67*).

Several simple benzoquinones are known only in a reduced form, usually as a glucoside, but there are scattered references (*68*) to the occurrence of hydroquinone, and also catechol, in the free state, in the leaves of several plants. These are possibly derived from gentisic (*69*) and protocatechuic (*68*) acids, respectively, which occur widely. Arbutin (LV) (hydroquinone monoglucoside) occurs in several families, more especially in the Ericaceae, sometimes accompanied by its methyl ether (methylarbutin). Usually it is present in the leaves, but in *Geum rivali* Bourg. it occurs also in the roots (*70*), and in *Rhododendron pontica* Linn., in the flowers, leaves, and stems (*71*). The genus *Pyrola* is of particular interest; *P. rotundifolia* Linn. produces arbutin (*72*), homoarbutin (LVI) (*73*), and the naphthoquinone chimaphilin (XXXVIII), and pyrolatin (LVII) has been isolated from the leaves of *P. japonica* Sieb. (*74*). The latter has a C_{10} terpenoid side chain. 2,6-Dimethoxybenzoquinone has been found in several dicotyledonous plants, but in wheat it is present in a reduced form. Both 2-methoxy- and 2,6-dimethoxyquinol occur in wheat germ and wheat bran (*75*); the former has been isolated from the germ as its monoglucoside (*76*).

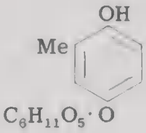
The most interesting and most important benzoquinones produced by higher plants are the ubiquinones (coenzymes Q) and plastoquinone (*76a*), which may occur generally in green leaves. Plastoquinone (2,3-dimethyl-5-solanesyl-1,4-benzoquinone, previously Q_{254}) (LXVII) was first isolated by Kofler (*78*) from alfalfa and from horse chestnut leaves and was also identified spectroscopically in the leaves of oats, ivy, and stinging nettle. Recent investigations in the Merck (*79*) and Hoffman-La Roche (*80, 81*) laboratories have established the structure of this quinone, which has a C_{45} polyisoprenoid side chain and is closely related to the ubiquinones. Kofler and co-workers (*80*) have isolated plastoquinone from the leaves of some twenty plants (oak, ash, beech, sycamore, willow, fig, sunflower, etc.), and Crane (*82*), using spinach, has shown that it is concentrated in the chloroplasts. In general, it has been detected in the photosynthetic tissues of all higher plants examined but is not limited exclusively to chlorophyll-containing tissues. The work of Crane (*82*) revealed that plastoquinone is present in the variegated leaves of the monocotyledon *Pandanus vetchii* (Pandanacea), but the amount present in the green portions (0.0173 mg. per gram fresh weight) is ten times that in the white portions (0.0017 mg. per gram). Small amounts also exist in corn roots (*Zea mays*) and cauliflower buds, which are capable of developing chloroplasts, and the quinone is possibly present in the proplastids of these

Structure and Distribution of Benzoquinones and Benzoquinols in Flowering Plants[a]



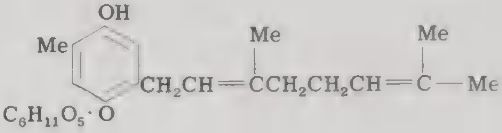
Arbutin (*68*)
(Ericaceae,
Leguminosae,
Rosaceae,
Vacciniaceae,
Proteaceae)

(LV)



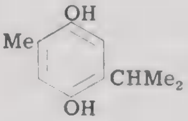
Homoarbutin (*73*)
(Ericaceae)

(LVI)



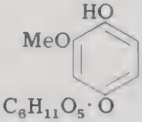
Pyrolatin (*74*)
(Ericaceae)

(LVII)



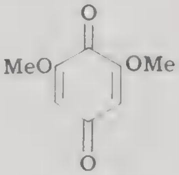
Thymoquinol
(Labiatae,
Umbelliferae)

(LVIII)



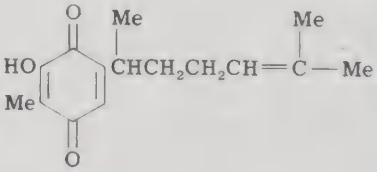
(Gramineae)

(LIX)



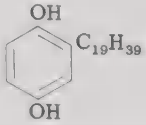
[Ranunculaceae,
Simarubeae (*77*)
Meliaceae (*77*)]

(LX)



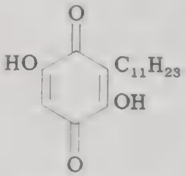
Perezone
(Compositae)

(LXI)



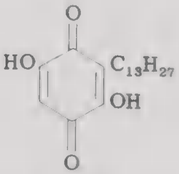
[Anacardiaceae (*64*)]

(LXII)



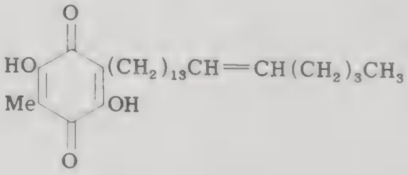
Embelin
(Myrsinaceae)

(LXIII)



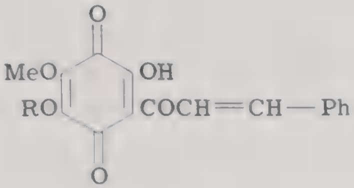
Rapanone
(Myrsinaceae,
Geraniaceae)

(LXIV)



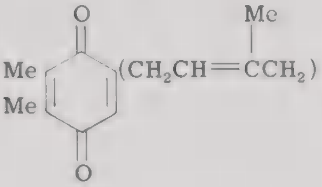
Maesaquinone
(Myrsinaceae)

(LXV)



Pedicinin (R=H)
Methylpedicinin (R=Me)
(Gesneraceae)

(LXVI)



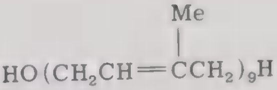
Plastoquinone
(Green leaves)

(LXVII)

[a] For references to individual spp. see (*1*) except where indicated.

tissues (*83*). The tissues of white potatoes, which do not produce chlorophyll, do not contain plastoquinone (nor do the photosynthetic bacteria *Rhodospirillum rubrum* and *Chromatium* spp.). Coenzyme Q is present in all these tissues, both white and green, but is confined to the mitochondria whereas plastoquinone is a chloroplast (or potential chloroplast) constituent. Plastoquinone is not involved in respiratory electron transport (the function of coenzyme Q), but takes part in photosynthetic electron transport (p. 648). At present only one plastoquinone is known, but analogy with the ubiquinones and the vitamin K_2 series suggests that this may be only the first of a new family of quinones, and there are already indications of this (*56*).

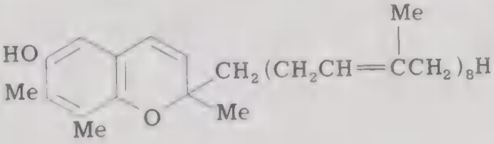
Rowland (*84–87*) has recently isolated from tobacco leaf a number of long-chain polyisoprenoid compounds including the alcohol solanesol (LXVIII), and several esters. Folkers *et al.* (*88*), and also Isler *et al.*



$HO(CH_2CH{=}\overset{\displaystyle Me}{\overset{|}{C}}CH_2)_9H$

Solanesol

(LXVIII)



Solanachromene

(LXIX)

(*81*) have shown that it is a C_{45} molecule [not C_{50} as originally proposed (*84*)] and both groups have used it to synthesize plastoquinone

(*81*, *89*) *in vitro*. Another related compound found in tobacco leaf is solanachromene (LXIX) (*86*), a cyclic isomer of plastoquinone.

Most higher plants examined so far contain coenzyme Q* but it appears to be absent (or present in a concentration too low to be detectable) from lettuce, carrots, green peas, cabbage, and potatoes, according to Folkers *et al.* (*82a*). However, Lester and Crane (*83*) detected coenzyme Q_{10} in potato tubers, and also in sweet potato roots, spinach and alfalfa leaves. [Crystalline Q_{10} has since been isolated from alfalfa (*76a*).] They also detected unidentified coenzymes Q in young corn shoots and roots, cauliflower buds, cotton seed, and wheat germ oil, but not in olive or linseed oil. According to Folkers the isoprenolog in wheat germ oil and in corn oil is Q_9, whereas in soybean oil it is Q_{10}. In a detailed study of spinach leaves and corn seedlings, Crane (*82*) found that coenzyme Q occurred in both white and green tissues, concentrated in the mitochondria (*82*). This suggests that coenzyme Q is involved in mitochondrial electron transport in plants, as in animals. In keeping with the respiratory function of these quinones is the recent identification of ubiquinone* in the highly aerobic spadix of *Arum maculata* Linn. (*90*).

b. Gymnospermae. In addition to the many terpenes that occur in the Cupressaceae there are a number of aromatic compounds, including two quinones,† which are closely related to the monocyclic monoterpenes. Thus Grimal (*91*) found carvacrol (LXX), thymoquinol and thymoquinone (LXXII), in the heartwood of *Tetraclinis articulata* (Vahl) Masters, and Zavarin and Anderson (*92–94*) have isolated the following compounds from the wood of the incense cedar, *Libocedrus decurrens* Torrey: carvacrol, thymoquinol and both its monomethyl ethers—*p*-methoxycarvacrol and *p*-methoxythymol (LXXI)—thymoquinone, libocedrol (LXXIII), heyderiol‡ (LXV), and 3-libocedroxythymoquinone (LXXIV). Thymoquinone and the monocyclic phenols have fungicidal properties, which are no doubt partly responsible for the decay resistance of the heartwoods.

In the laboratory, libocedrol can be prepared by oxidation of *p*-methoxythymol with alkaline ferricyanide (*94*) and both libocedrol and heyderiol are formed by a similar oxidation of a mixture of *p*-methoxythymol and *p*-methoxycarvacrol (*93*). Zavarin (*93*) has also

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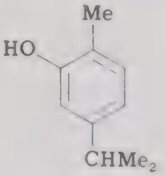
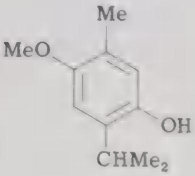
[*] Further discussion of these compounds, and their formulas, are given on page 692.

[†] Now four: 3-hydroxy-, and 3,6-dihydroxythymoquinone have recently been isolated from the heartwood of *Juniperus chinensis* (*90a*).

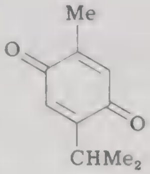
[‡] According to Li (*95*), *L. decurrens* Torrey is now *Heyderia decurrens* Torrey.



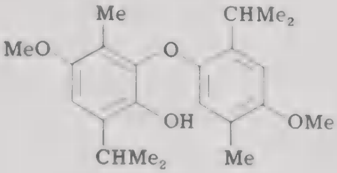
(LXX)



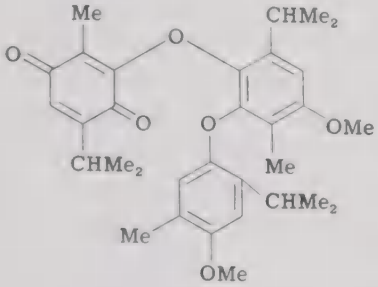
(LXXI)



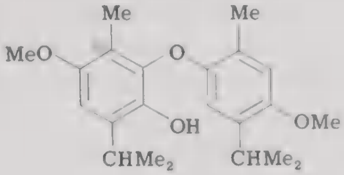
(LXXII)



(LXXIII)



(LXXIV)



(LXXV)

shown that a ferricyanide oxidation of libocedrol gives a mixture containing 3-libocedroxythymoquinone (46%). These one-electron transfer reactions produce mesomeric phenoxyl radicals initially, which can dimerize in various ways, and similar radical coupling oxidations must take place enzymatically *in vivo*. Libocedrol could arise by hydrogen abstraction from *p*-methoxythymol followed by dimerization, and a similar coupling of radicals derived from *p*-methoxythymol and *p*-methoxycarvacrol would give heyderiol; the libocedroxyl radical formed by further hydrogen abstraction could then give rise to libocedroxythymoquinone by reaction with thymoquinone, by coupling with *p*-methoxythymol or thymoquinol radicals, or by more complex reactions as in the *in vitro* synthesis. The formation of lignin and other complex products in conifers by oxidative coupling of phenols is a common phenomenon (*96*). It has been established that all the oxidation products mentioned above occur in the wood *in situ*, but further oxidation takes place during air seasoning and during the isolation procedures. When acetone-extracted cedar sawdust was impregnated with a mixture of libocedrol, *p*-methoxythymol, and thymoquinone and exposed to the air for three months, 3-libocedroxythymoquinone was formed (*92*).

Plastoquinone also appears in the Gymnospermae. It was detected spectroscopically in pine needles in the original work of Kofler (*78*) and was isolated recently from the leaves of the maidenhair tree (Ginkgoaceae) (*80*).

2. *Fungi*

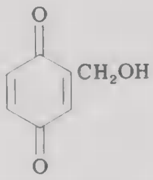
a. Benzoquinones. Almost all the fungal benzoquinones are produced by Hyphomycetes or Basidiomycetes, the majority having a very limited distribution. Oosporein (LXXXVI) and the coenzymes Q (p. 692) occur more widely. In no case has the same quinone been obtained from both higher and lower fungi, although quinones of similar structure occur in both classes. All the terphenylquinones are found in Basidiomycetes with the exception of the hyphomycetous pigment volucrisporin (LXXXIX), and all the simpler quinones are formed by Hyphomycetes save (LXXXVII), which is produced by two Basidiomycetes when grown on corn-steep-glucose media. Thus, with the exceptions noted, the fungal benzoquinones can be divided into (*a*), simple derivatives of benzoquinone and toluquinone, (LXXVI) to (LXXXIV), and the related diquinones (LXXXV) and (LXXXVI), which are produced by Hyphomycetes on synthetic media, and (*b*), the terphenylquinones, (LXXXVII) to (XCII), obtained from Basidiomycetes growing wild.

The simplest fungal quinone is gentisylquinone (LXXVI), which appears as a deep-violet quinhydrone, $(C_7H_8O_3)_3$, $C_7H_6O_3$, in cultures of *Penicillium patulum* Bainier (*97*). It is the only benzoquinone bearing a hydroxymethyl group, a common structural feature in the anthraquinone series. In recent work with a nonpigmented mutant strain of *P. patulum*, Tanenbaum and Bassett (*98*) have isolated several simple aromatic compounds including gentisyl alcohol [the quinol of (LXXVI)], gentisaldehyde, and gentisic acid. Auroglaucin and flavoglaucin (LXXXII) are substituted gentisaldehydes (orange and yellow, respectively) formed by numerous species of the *Aspergillus glaucus* series (along with anthraquinone pigments) (*99*). Another highly colored quinol is 2,4,5-trihydroxyphenylglyoxylic acid, a bright red metabolic product of *Polyporus tumulosus* Cooke (*100*).

Spinulosin (LXXXIV), the first fungal quinone to be identified, was originally obtained from cultures of *Penicillium spinulosum* Thom by Birkinshaw and Raistrick (*101*) and was the forerunner of numerous quinones which were isolated later in their laboratory. It is also formed by *Aspergillus fumigatus* Fresenius (*102*), another strain of which produced the closely related quinone fumigatin (LXXXIII) (*103*). Like several of the simple quinones shown on page 656, spinulosin shows fairly high antibiotic activity. The diquinones oosporein (LXXXVI) and phoenicin (LXXXV) have a structural relationship like that of spinulosin and fumigatin, but have not been found in the same genus. *Penicillium rubrum* O. Stoll (*104*) elaborates both phoenicin and its leuco compound, and the strain of *Aspergillus fumigatus* which synthesizes fumigatin, also produces the corresponding quinol, the quinone: quinol ratio varying according to the stage of development of the organism. An unusual metabolite is formed by a *Gliocladium* species (probably *G. roseum* Bainier) (*105*) when grown on Raulin-Thom solution; in addition to an orange quinone, aurantiogliocladin (LXXX), and its dark red quinhydrone, rubrogliocladin, it yields a colorless reduction product gliorosein (LXXXI), isomeric with aurantiogliocladin quinol. This is the only naturally occurring quinol in the benzene series that is known in the tautomeric diketo form. [There is also one example in the naphthoquinol series (XLVII).] Another unusual derivative of 2,3-dihydrobenzoquinone is the optically active epoxide terreic acid (LXXIX), a metabolic product of *Aspergillus terreus* (*106*).

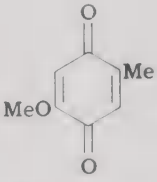
The terphenylquinones (LXXXVII) to (XCII) are a unique group of natural products, confined to the Agaricales (excepting volucrisporin, as already mentioned) and certain lichens. They are all derivatives of 2,5-diphenylbenzoquinone with a varying pattern of hydroxylation reminiscent of flavonoids and are mostly dark brown in color. The bronze pigment polyporic acid (LXXXVII) constitutes 23% of the dry weight of *Polyporus rutilans* (Pers.) Fries (*107*). Thelephoric acid, which is accompanied by anthraquinone pigments in *Polystictus versicolor* (*108*) was thought formerly to be a phenanthrenequinone (*109*), but work in several laboratories has shown that it belongs to the terphenylquinone group and Gripenberg (*110*) has very recently established the pentacyclic structure (XCII) by synthesis. Volucrisporin (LXXXIX), the only quinone of this group found in a lower fungus, differs from the others in the absence of oxygen functions on the quinone ring and in the rare existence of *m*-hydroxyphenyl groups (*111*). Both the red pigment and its quinol are present in the metabolism solution of *Volucrispora aurantiaca* Haskins. Unusual reduction products are also a feature of these quinones. Leucomelone (XC), the brown pigment present in the black edible mushroom *Polyporus leucomelas* Pers. ex Fr., is accompanied by a colorless compound, protoleucomelone, which is the hepta-acetate of leucomelone quinol (*112*). Aurantiacin, the dark red 3,6-dibenzoate of atromentin (LXXXVIII) is found in *Hydnum aurantiacum* Batsch, together with its leucodibenzoate and theleporic acid (*113*). Atromentin itself occurs mainly in a reduced form in *Paxillus atrotomentosus* (Batsch) Fr., for only the exterior of the fungus is colored. As the quinone was isolated by alkaline extrac-

Fungal Benzoquinones[a]



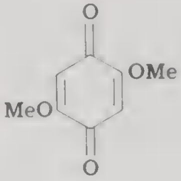
Gentisylquinone
(*Penicillium* spp.)

(LXXVI)



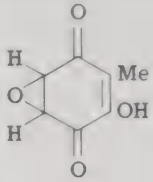
[*Coprinus* and
Lentinus spp.
(Agaricaceae)]

(LXXVII)



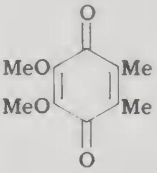
(*Polyporus* spp.)

(LXXVIII)



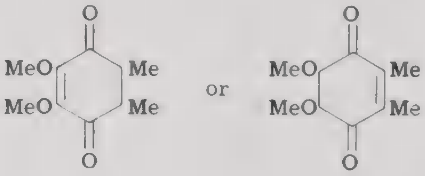
Terreic acid (*106*)
(*Aspergillus* sp.)

(LXXIX)



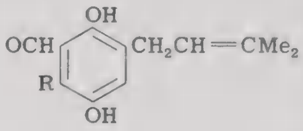
Aurantiogliocladin
(*Gliocladium* sp.)

(LXXX)



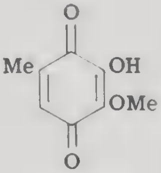
Gliorosein
(*Gliocladium* sp.)

(LXXXI)



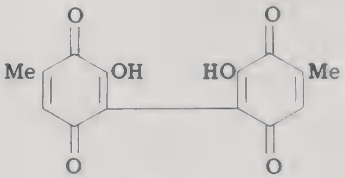
Auroglaucin
[R=—$(CH=CH)_3Me$]
Flavoglaucin
[R=C_7H_{15}—]
(*Aspergillus* spp.)

(LXXXII)



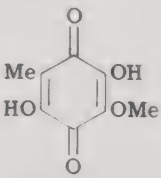
Fumigatin
(*Aspergillus* spp.)

(LXXXIII)



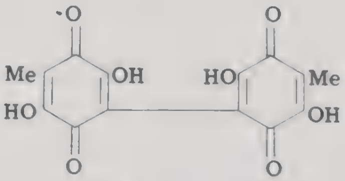
Phoenicin
(*Penicillium* spp.)

(LXXXV)



Spinulosin
(*Penicillium* and
Aspergillus spp.)

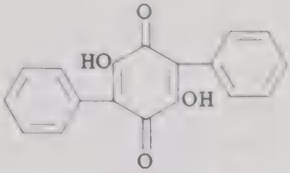
(LXXXIV)



Oosporein
[*Oospora*, *Acremonium*,
Verticillium and
Chaetomium spp. (*104a*)]

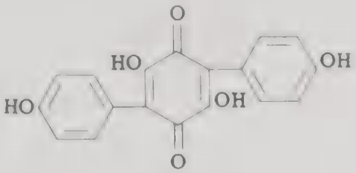
(LXXXVI)

Fungal Benzoquionones[a] (continued)



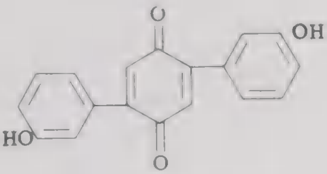
Polyporic acid
[*Polyporus* and
Peniophora spp.
(Thelephoraceae)]

(LXXXVII)



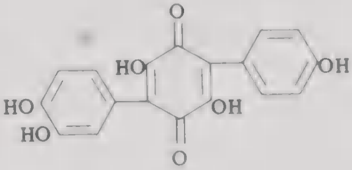
Atromentin[b]
[*Paxillus* sp.
(Agaricaceae)]

(LXXXVIII)



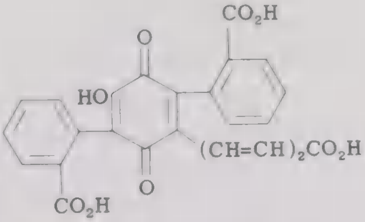
Volucrisporin (*112*)
(*Volucrispora* sp.)

(LXXXIX)



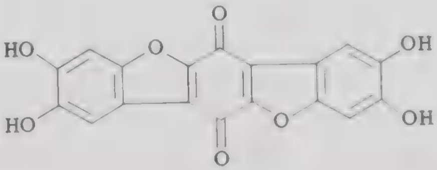
Leucomelone
(*Polyporus* sp.)

(XC)



Muscarufin[c]
[*Amanita* sp.
(Agaricaceae)]

(XCI)



Thelephoric acid
[*Thelephora* and *Hydnum* spp.,
Cantharellus sp (Agaricaceae),
Polystictus sp. (Polyporaceae)]

(XCII)

[a] For references to individual species see (*1*), except where indicated.

[b] Aurantiacin (*Hydnum* sp.) is the 3,6-dibenzoate (*114*).

[c] See text.

tion, it is possible that it too is present as an acylated derivative of the leuco compound, and not simply as atromentin quinol. The structure proposed (*114*) for muscarufin (XCI) is very dubious. A recent re-examination by Eugster (*114a*) of the red skins of *Amanita muscaria* (Linn.) Fries showed that several labile, non-quinonoid, pigments were present, and the original work of Kögl and Erxleben (*114*) could not be repeated.

Coenzymes Q* occur in the fungi (*83*) as in other divisions of the plant kingdom but are not ubiquitous. Three have been isolated from Ascomycetes: Q_7 and Q_9 from *Torula utilis* (*115, 116*), Q_6 from *Saccharomyces cerevisiae* (*117*). The latter has also been identified in *S. cavalieri* and *S. fragilis*, but it is not formed under anaerobic conditions, which is consistent with its respiratory function. Coenzyme Q_{10}, the isoprenolog commonly found in higher animals and plants, is also present in *Neurospora crassa;* in the phycomycete *Mucor corymbifer* it is replaced by Q_9. This family of quinones appears to be absent from the Basidiomycetes,† but in four species (*Psalliota campestris, Coprinus atramentarius, Ustilago zea,* and a *Polyporus* sp.) examined by Lester and Crane (*83*), a new lipid-soluble quinone was detected. This new substance, basidioquinone, can be recognized spectroscopically by a broad band at 255 mμ, which disappears on reduction with potassium borohydride and is replaced by a peak at 295 mμ. Nothing is known of the structure of this quinone which occurs in highest concentration in actively growing fungal tissue.

b. Naphthoquinones. The few known fungal naphthoquinones, (XCIII) to (XCVII), have all been obtained in laboratory culture on synthetic media. They mostly show weak antibiotic properties, which attracted attention and led to their chemical investigation. There is one example (XCIII) in the Agaricaceae and another in *Aspergillus,* but otherwise the naphthoquinones have little botanical connection with the fungal benzoquinones. The two *Fusarium* quinones, (XCVI) and (XCVII), are closely related, and indeed both have been isolated from the same species. [The red pigment bostrycoidin, $C_{17}H_{11}O_6 \cdot OCH_3$, a metabolite of *F. bostrycoides* Wr. and Rkg. may belong to this group (*118*).] The hemiacetal structure of fusarubin is rare in natural products (apart from the sugars), and this quinone is also unusual in that it occurs in cultures of *F. solani* (Mart.) App. and Wr. partly in a water-soluble reduced state. The corresponding water-soluble pigment fusarubinogen, has been isolated, and was found to be a sulfuric ester of fusarubin (*119*). The structure postulated for mollisin (XCV) is not established on the present evidence (*120*), and further investigation may show it to be more closely related to javanicin, but it is noteworthy that this pigment was obtained from an inoperculate discomycete. The orange pigment mycochrysone, $C_{20}H_{12}O_7$, also produced by an inoperculate discomycete, is a more complicated molecule, which appears to be, in part, a *peri*-hydroxy-β-naphthoquinone (*121*). The pigments of the Discomycetes have been somewhat neglected and may repay further study.

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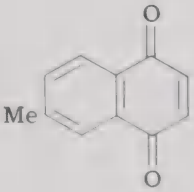
* These quinones are discussed more fully on page 692.

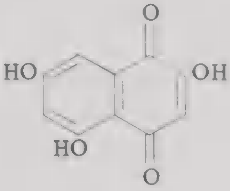
† See however, reference *117a*.

Fungal Naphthoquinones[a]



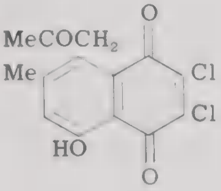
[*Marasmius* sp.
(Agaricaceae)]

(XCIII)



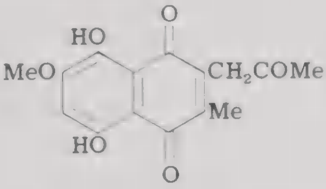
Flaviolin
(*Aspergillus* sp.)

(XCIV)



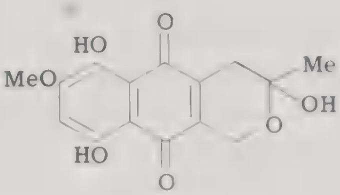
Mollisin
(*Mollisia* sp.)

(XCV)



Javanicin
(*Fusarium* spp.)

(XCVI)



Fusarubin
(*Fusarium* spp.)

(XCVII)

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[a] For references to individual species see (*1*).

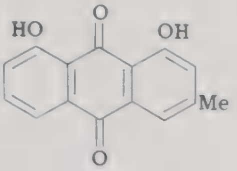
c. Anthraquinones. This is the largest group of fungal quinones, all of which have been found in Hyphomycetes except boletol (CXII), and phomazarin (CXI), which are also exceptional in structure. *Penicillium, Aspergillus*, and *Helminthosporium* species are the main sources (see pp. 661–663), a number of quinones appearing in more than one genus. *Helminthosporium gramineum* Rabenhorst grown on Czapek-Dox-glucose solution has been known to yield a mixture of helminthosporin (CVII) and catenarin (CVIII), amounting to as much as 30% of the dry weight of the mycelium (*122*). Emodin (XCIX; R = Me), probably the most widely distributed anthraquinone, has been found also in Basidiomycetes [*Polystictus versicolor* (*108*) and *Cortinarius sanguineus* (Wulf) Fries (*123*)], an ascomycete [*Penicilliopsis clavariaeformis* Solms-Laubach (*124*)], and a pyrenomycete [*Chaetomium affine* Corda (*125*)], the two latter grown on synthetic media. Three of the *Helminthosporium* pigments catenarin, cynodontin, and helminthosporin, occur also in an ascomycete, *Deuterophoma tracheipila* Petri (*126*). Several other anthraquinones are known outside of the Hyphomycetes, an interesting example being endocrocin (CVI), first known as a lichen pigment and recently found in ergot (*Claviceps purpurea,* a pyrenomycete) (*128*). The complex mixture of metabolites produced by the latter includes two anthraquinones, clavoxanthin (= endocrocin) and clavorubin, which is not yet identified.

In structure, the fungal anthraquinones form a very compact group. Nearly all are of the emodin type, the differences being mainly in the pattern of hydroxylation: relative to emodin, hydroxyl groups may be absent, as in chrysophanol (XXVI) and the hydroxy-methylanthraquinone (CX) [both of which are formed by *Pachybasium candidum* (Sacc.) Peyronel (*129*)] or, additional hydroxyl groups may be introduced *ortho* or *para* to an existing one, as in catenarin (CVIII), or, as in islandicin (XCVIII), both variations may be effected. Versicolorin (CV) does not fit into this pattern, but the structure of this quinone requires confirmation (*130*). Boletol is most unusual in having a 1,2,4 arrangement of hydroxyl groups like that found in the rubiaceous quinones, and a carboxyl group in an alpha position. When the flesh of some *Boletus* spp. is cut and exposed to air, it turns blue owing to the formation of the diquinone (CXIII) by enzymatic oxidation. *B. subtomentosus* Linn. and *B. chrysenteron* Bull. both contain boletol but do not turn blue when broken, which Bertrand (*131*) ascribed to the absence of laccase. The azaanthraquinone phomazarin (CXI) is a metabolic product of *Phoma terrestris* Hansen and is unique in structure. As already noted, the formation of several related pigments by a single fungus is quite common, and the amount is sometimes substantial. In this respect the most remarkable organism is *Penicillium islandicum,* from which the following pigments, amounting to approximately 20% of the dry weight of the mycelium, have been obtained: islandicin, chrysophanol, emodin, endocrocin, and catenarin, besides several dianthraquinones. No anthraquinols have been found in fungi, which is hardly surprising, but anthrones have been isolated. Several members of the *Aspergillus glaucus* series produce physcion (CII) and erythroglaucin (CIII) (in addition to auroglaucin and flavoglaucin), and five species yielded, in addition, the two anthrones of physcion, which have also been found in higher plants (*132*).

The dianthraquinones, first discovered in Raistrick's laboratory and extensively studied in recent years by Shibata, are closely related to the foregoing simpler compounds. Thus skyrin (CXIV) is diemodin, and iridoskyrin (CXV) is diislandicin, and all four pigments are produced by *P. islandicum* together with the diquinones hydroxyskyrin and pigment C (CXIV), the closely related yellow pigments rubroskyrin (CXVI) and luteoskyrin (CXVIII), and the other simple qui-

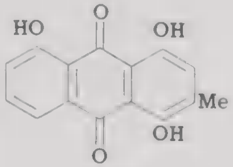
Fungal Anthraquinones [a]

In *Penicillium*



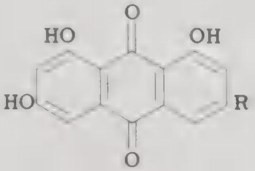
Chrysophanol
[Also in *Pachybasium*,
Chaetomium (125), and
Sepedonium (124) spp.]

(XXVI)



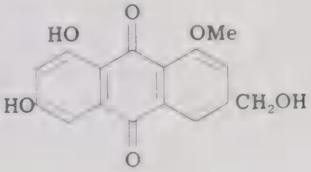
Islandicin

(XCVIII)



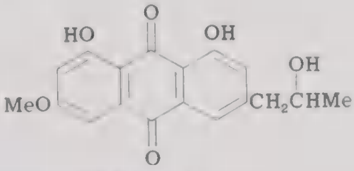
Emodin (R = Me) *(140)*
Citreorosein (R = CH_2OH)
Emodic acid (R = CO_2H)
[Emodin also occurs in
Chaetomium (125), *Cladosporium (139)*,
Cortinarius (Agaricaceae),
Polystictus (Polyporaceae) *(108)*, and
Penicilliopsis (Eurotiales) *(124)* spp.]

(XCIX)



Roseopurpurin

(C)



Nalgiovensin
(Nalgiolaxin is the
5- or 8-chloro derivative)

(CI)

nones referred to above. The diquinones are possibly formed by radical coupling of the monoquinones* or their anthrones [for examples of this type of oxidative dimerization see (*133*)], and the reverse reaction is readily achieved *in vitro* by reduction with sodium dithionite. Rugulosin (CXVII) was first obtained from *P. rugulosum* Thom [forming 20% of the weight of the dry mycelium in one strain (*134*)] and it occurs,

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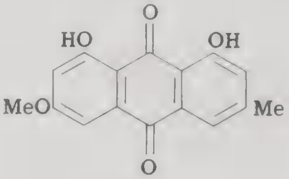
* See, however, page 705.

Fungal Anthraquinones [a] (continued)

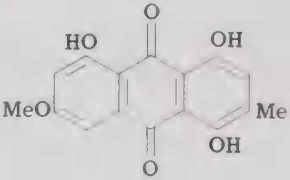
In *Aspergillus*



Physcion

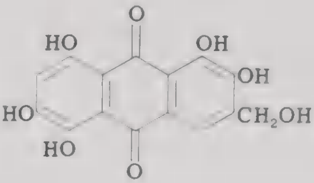
(Also in *Penicillium* sp.)

(CII)



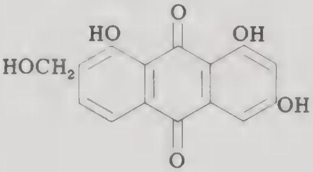
Erythroglaucin

(CIII)



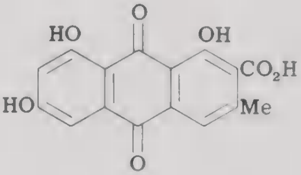
Asperthecin [b]

(CIV)



Versicolorin [e]

(CV)

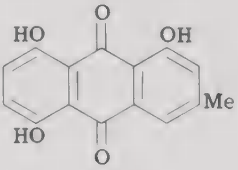


Endocrocin

[Also in *Penicillium (127)* and *Claviceps* (Hypocreales) spp. *(128)*]

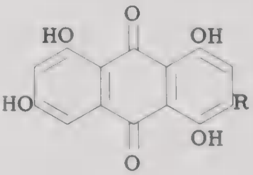
(CVI)

In *Helminthosporium*



Helminthosporin [c]

(CVII)

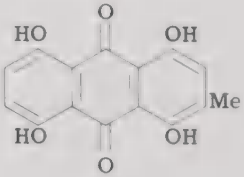


Catenarin [c] (R = Me)

(Also in *Penicillium* spp.)

Tritisporin (R = CH_2OH)

(CVIII)



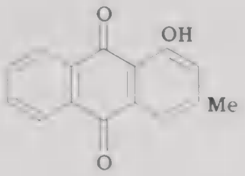
Cynodontin [c]

(CIX)

usually with skyrin, in several other fungi. The compounds luteoskyrin and rugulosin are not strictly anthraquinones but are readily converted thereto by dehydration; e.g., on treating rubroskyrin with sulfuric acid it is converted into iridoskyrin, a process of aromatization (*135*). On exposure to light in acetone solution luteoskyrin is transformed into a purple compound, lumiluteoskyrin (purpurmycelin), which is a true quinone (*136*). Anthrones are also known in this series, *Penicilliopsis clavariaeformis* Solms-Laubach providing both skyrin and its dianthrone, penicilliopsin (5,5′-diemodin-9-anthrone) (*124*). A serious problem

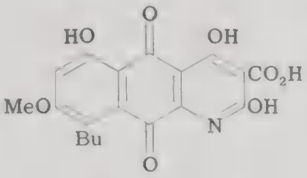
Fungal Anthraquinones[a] (continued)

From other sources



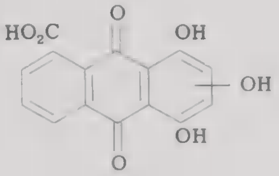
(*Pachybasium* sp.)

(CX)



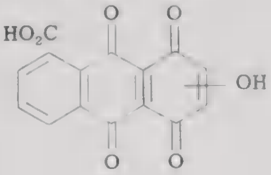
Phomazarin[d]
[*Phoma* sp.
(Sphaeropsidales)]

(CXI)



Boletol
[*Boletus* spp.
(Agaricales)]

(CXII)



(CXIII)

[a] For references to individual species see (*1*), except where indicated. Macrosporin, produced by "*Macrosporium porri* Elliott," is 2,8-dihydroxy-6-methoxy-3-methylanthraquinone (*128a*).

[b] (CIV) is one of two possible structures.

[c] Also occurs in a *Deuterophoma* (*126*) and *Phoma* (*126a*) sp. (Sphaeropsidales).

[d] (CXI) is one of two possible structures.

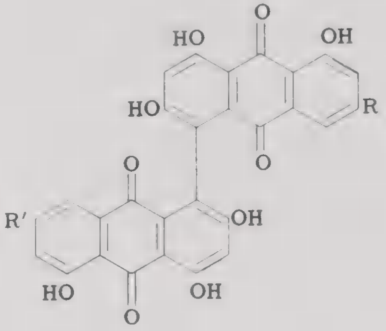
[e] Structure doubtful (*127a*).

arose in Japan a few years ago through the importation of a large amount of moldy rice infected with *P. islandicum*. It was found that luteoskyrin was one of the toxic principles produced, causing severe damage to the liver when administered to animals (*137*). Skyrin is one of the toxins elaborated by *Endothia parasitica* (Murr.) Anderson and Anderson (chestnut blight) (*138*).

3. *Lichens*

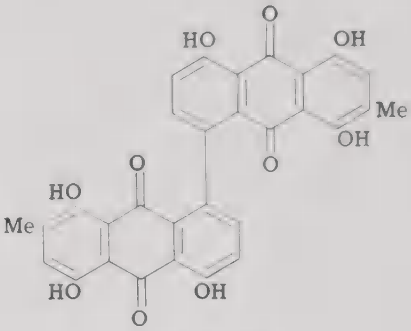
The lichen quinones show a marked resemblance to the fungal quinones as would be expected, but are far less numerous. A few are common to both lichens and fungi, and indeed the first definite evidence

Fungal Dianthraquinones and Related Compounds[a]



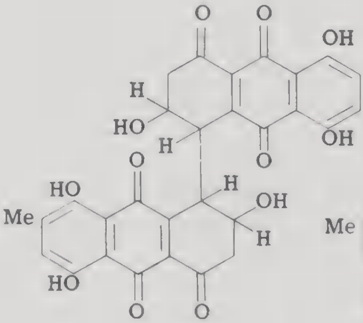
Skyrin (R = R′ = Me)
[*Penicillium*, *Sepedonium (124)*, *Penicilliopsis (124)*, and *Endothia* (Sphaeriales) spp.]
Hydroxyskyrin (R = Me; R′ = CH_2OH)
[*Penicillium* and *Endothia* spp. *(141)*]
Pigment C (R = R′ = CH_2OH)
(*Penicillium* sp.)

(CXIV)



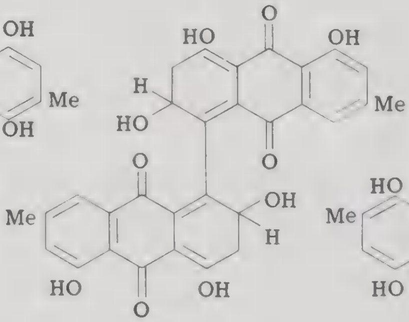
Iridoskyrin
(*Penicillium* sp.)

(CXV)



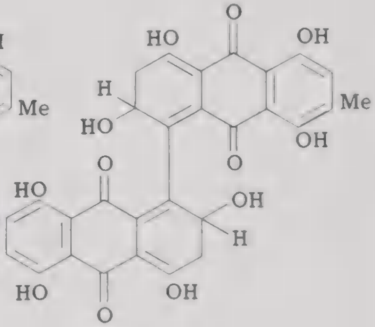
Rubroskyrin
(*Penicillium* sp.)

(CXVI)



Rugulosin
[*Penicillium*, *Sepedonium (124)*, and *Endothia* spp.]

(CXVII)



Luteoskyrin
[*Penicillium* and *Mycelia sterilia* spp. *(142)*]

(CXVIII)

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[a] For references to individual species see (*1*), except where indicated.

that lichen metabolites are formed by the fungal half of the symbiont was the isolation, by Raistrick (*143*), of the well-known lichen pigment physcion (CXX; R = Me) from the fungus "*Aspergillus ruber* (Mangin) Raper and Thom." This received powerful support from the work of Castle and Kubsch (*144*), who showed that the isolated fungal component of *Cladonia cristatella* could still produce rhodocladonic acid

(CXXI) and other lichen acids, when grown on synthetic media. Hess (*145*), however, was unable to confirm these results; in his experiments with mycobionts isolated from *Cladonia, Parmelia,* and other species, the lichen acids characteristic of these species were not produced and only simple phenolic acids, e.g., orsellinic and haematommic acids could be detected. He concluded therefore that the algal component plays a part in the biogenesis of the more complex lichen metabolites by the provision of some "chemischen Faktoren." This factor is evidently glucose. The growth medium used by Hess contained malt extract and agar, but no simple carbohydrate material was added. Scott (*145a*) has found that the isolated mycobiont of *Xanthoria parietina* produces physcion only when it is supplied with glucose, in agreement with earlier work (*145b*). Castle and Kubsch also appear to have used glucose in their media,[*] and presumably a simple sugar photosynthesized in the algal cells is the source of the aromatic compounds produced by the fungal component of the living lichen.

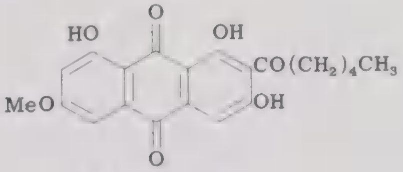
So far no naphthoquinones have been found in lichens, and only two benzoquinones and seven anthraquinones are known. This corresponds roughly to the proportions in which each type occurs in the fungi. The benzoquinones, polyporic acid and theleporic acid, found in Stictaceae, occur also in the Basidiomycetes, but there appears to be no possible botanical relationship between the latter and these Ascolichenes. The terphenylquinones are closely related to the pulvinic acid pigments, which are confined to lichens (Stictaceae and related families). Polyporic acid, theleporic acid, pulvinic acid (CXXIV), and pulvinic acid

Lichen Quinones

Peltigerales

Polyporic acid
[*Sticta* spp. (Stictaceae)]
(LXXVII)

Theleporic acid
[*Lobaria* spp. (Stictaceae)]
(XCII)



Solorinic acid
[*Solorina* sp.
(Peltigeraceae)]
(CXIX)

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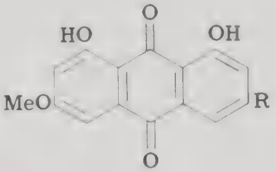
[*] Castle and Kubsch give very little detail in their preliminary paper and do not actually mention glucose, but see reference *145c*.

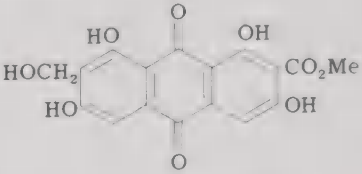
Parmeliales



Physcion (= Parietin) (R = Me)
[*Teloschistes* and *Xanthoria* spp.
(Teloschistaceae) *Caloplaca* and
Gasparrinia spp. (Caloplacaceae)]
Teloschistin (= Fallacinol)
(R = CH_2OH) (*Teloschistes*
and *Xanthoria* spp.)
Fallacinal (R = CHO)
(*Xanthoria* sp.)
Parietinic acid (R = CO_2H)
(*Xanthoria* sp.) (*149*)

(CXX)

Cladoniales



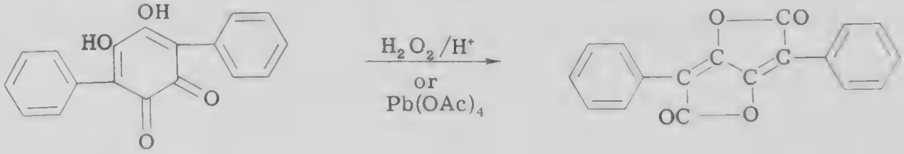
Rhodocladonic acid
[*Cladonia* spp. (Cladoniaceae)]

(CXXI)

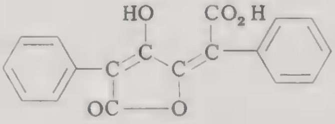
Endocrocin [*Nephromopsis* sp.
(Parmeliaceae)]

(CVI)

lactone (CXXIII) are products of *Sticta coronata* (*146*). Pigments of both types yield similar degradation products, and almost certainly have



(CXXII) (CXXIII)



Pulvinic acid

(CXXIV)

the same precursors. Where they occur together it is conceivable that the pulvinic acid compounds are derived from the quinones, as the former can be converted into the latter *in vitro* by oxidation with hydrogen peroxide under acid conditions, or, more efficiently, using lead tetraacetate. Rewriting polyporic acid in the *o*-quinonoid form (CXXII), it can be seen that the dicarboxylic acid normally obtained by oxidation of a cyclic *o*-diketone with hydrogen peroxide will, in this case, form a dilactone (CXXIII) which is pulvinic acid lactone. The initial step in the lead tetraacetate oxidation is probably dehydrogenation of the two hydroxyl groups in (CXXII), for which enzymatic analogies exist (*147*) (a possible mechanism is given on page 699). The other pulvinic acid coloring matters are esters or amides of (CXXIV), or derivatives in which hydroxyl (or methoxyl) groups are present in a benzene ring, reminiscent of the terphenylquinone structures (*68*).

The best-known lichen quinones are the anthraquinones, notably physcion (parietin to lichenologists), which colors the thalli of numerous Parmeliales bright yellow. Closely related pigments, differing only in the state of oxidation of the side chains occur together: physcion, fallacinol, and fallacinal in *Xanthoria fallax* (Hepp.) Arn. (*148*), and these three together with parietinic acid in *Xanthoria parietina* (L.) Beltram (*149*). Endocrocin, found in a Japanese lichen, is also a fungal quinone of the emodin type, but the other two red quinones solorinic acid (CXIX) and rhodocladonic acid (CXXI) have a modified structure. Solorinic acid occurs on the underside of *Solorina crocea* (L.) Ach., and rhodocladonic acid is responsible for the red color of the apothecia of many *Cladonia* species. Again no botanical relationship is discernible between the *Penicillium* and *Aspergillus* sources of the emodin-type quinones and the lichens that produce similar compounds.

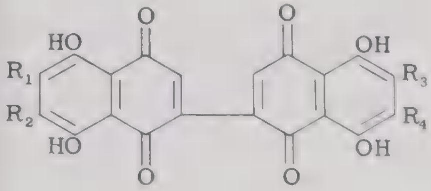
4. *Algae*

The existence of quinones in algae was unknown until quite recently. In their survey of the distribution of coenzymes Q, Lester and Crane (*83*) detected several quinones in algae by spectrometric and chromatographic methods, but none have been isolated as pure compounds. Coenzyme Q_9 appeared in a *Cladophora* sp. (Chlorophyceae), and Q_9 [or possibly Q_{10} (*150*)] in a *Fucus* sp. (Phaeophyceae). An unidentified coenzyme Q occurs in a *Polysiphonia* sp. (Rhodophyceae), but these quinones could not be detected in the one species of Cyanophyceae (*Anacystis nidulans*) examined. Plastoquinone was present in all these algae, and there is a considerable amount in *Chlorella pyrenoidosa* (Chlorophyceae) when grown under artificial culture conditions in the laboratory (*150*). (See also reference *56*.) Vitamin K was detected spectroscopically in the green and blue-green algae, but not in the red or brown. These are the only quinones to be found in marine life excepting the naphthoquinones in echinoids and holothuroids, and the anthraquinones in crinoids. There is no obvious connection between the plant and animal pigments.

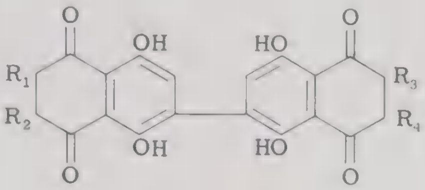
5. *Bacteria*

Although many bacteria produce pigments, few have been examined in detail. In recent years, the antibiotic properties of some of these have stimulated chemical investigation, and a number have now been recognized as quinones, several of a new type not found hitherto in nature. In contrast to the fungi, anthraquinones and benzoquinones are conspicuous by their absence. The Actinomycetales appear to be the most abundant source, but this may only reflect the attention they have received. However, the earliest record is somewhat dubious. In 1899, Beijerinck (*151*) claimed to have detected *p*-benzoquinone in cultures of *Streptomyces olivochromogenus* obtained from garden soil, and although this was not established, there was some indication of quinone formation, but only in media containing protein. In this respect, the organism behaves like other members of the "chromogenus" group of Streptomycetes in producing soluble brown pigments when grown on protein-containing media, and Beijerinck's quinone was most probably derived from tyrosine by enzymatic oxidation. According to this author, the quinone played an important part in humus formation, and the view is still held (*152*) that humic acids in soil are formed via quinones by polymerization and/or combination with protein, the quinones being produced by microorganisms from phenolic materials. The formation of quinones at an intermediate stage in the oxidative metabolism of phenols by bacteria is well established (*153*), and phenols may be available in soil as lignin degradation products or as leaf polyphenols, or they may be biosynthesized from carbohydrates by microorganisms.

The production of litmus-like pigments (i.e. pigments showing a red-blue, acid-base indicator change) by Actinomycetales has been observed for many years (*154*), and some at least are quinones. One of these is granaticin, $C_{22}H_{20}O_{10}$ (*155*), a deep-red antibiotic product of *Streptomyces olivaceus* (Waksman) Waksman and Henrici, which appears to be a polyhydroxypolycyclicquinone probably related to the pyrromycinones (see below); actinorhodin (*156*) is another. This latter coloring matter, obtained from *Streptomyces coelicolor* (Müller) Waksman and Henrici, is a derivative of dinaphthazarin (CXXV). Several derivatives of naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) have been isolated from the lower fungi, flowering plants, and sea urchins, but actinorhodin is the only dinaphthazarin, indeed the only dinaphthoquinone, isolated



Actinorhodin
(CXXV)



Protoactinorhodin
(CXXVI)

$$R_1R_2R_3R_4 = \begin{cases} 2CH_3 \\ 2CO_2H \\ C_8H_{16}O_2 \end{cases}$$

from natural sources. When S. *coelicolor* is grown on a medium containing glycerol and glycine, the mycelium formed contains 15% (dry weight) of pigment. This is normally isolated by alkaline extraction, which gives a blue solution, but if the mycelium is harvested and worked up under acid conditions, no blue color appears and a pale red substance, protoactinorhodin can be isolated (*157*). When this is aerated in alkaline solution, the initial yellow color quickly changes to blue, with the formation of actinorhodin. This, and other evidence, shows that protoactinorhodin, the precursor of actinorhodin, is a derivative of di-β-hydronaphthazarin (CXXVI). [Fusarubinogen (p. 658) probably exists in metabolism solutions as a β-hydronaphthazarin derivative.] Other litmus-like pigments reported are litmocidin (*158*) from "*Proactinomyces cyaneus-antibioticus*" [possibly a close relative of S. *coelicolor* (*159*)], streptocyanin (*160*) (from a *Streptomyces* sp., possibly S. *coelicolor*), rhodomycetin (*161*) produced by a mutant of S. *griseus*, coelicolorin (*162*) (also from S. *coelicolor*), mycorhodin (*162a*) and rubidin (*162b*) from unidentified Streptomycetes.

The most remarkable bacterial quinone pigments are naphthacene derivatives. They are produced by a number of Streptomycetes, found mainly in soil, where doubtless their antibiotic properties play an important role in the ecology of these microorganisms. It is significant that the only other naphthacene derivatives found in nature are the tetracycline antibiotics (e.g. CXXXI), which are also streptomycete metabolites. These red coloring matters (blue or violet in alkaline solution) occur in two forms: (*a*) water-soluble, amphoteric, glycosides [rhodomycins (*163*), pyrromycin (*164*), cinerubins (*165*), and rutilantins (*166*)[*]; and (*b*) water-insoluble, nonbasic aglycons [rhodomycinones (*163*, *167*) and pyrromycinones (*164*)]. The numerous rhodomycinones (see Table III), originally discovered by Brockmann and thought to be anthraquinones, are now known to be tetracyclic compounds (*167*). The other pigments in Table III are all closely related.

TABLE III
DISTRIBUTION OF NAPHTHACENEQUINONES IN STREPTOMYCETES

Species	Quinone	References
Streptomyces purpurascens[a]	Rhodomycin A	(*163, 167*)
	Isorhodomycin A	
	Rhodomycin B	
	Isorhodomycin B	
	β-Rhodomycinone	
	ε-Rhodomycinone	(*168b*)
	ε-Isorhodomycinone	(*168*)
	γ-Rhodomycinone	
	ζ-Isorhodomycinone	(*168a*)
Several unidentified spp.	η-, ζ-, and ε-Pyrromycinone	(*164, 168c*)
	Pyrromycin	
S. antibioticus *S. galileus* *S. niveoruber*	Cinerubin A Cinerubin B	(*165*)
Unidentified species	Rutilantins	(*166*)

[a] Six other rhodomycinones have been isolated from this species and three more detected by paper chromatography (*167*). Unidentified rhodomycins are also produced by *S. antibioticus* (*165*).

The secondary and tertiary alcoholic groups of ζ- (CXXVIII) and ε- (CXXVII) pyrromycinone are easily eliminated, and both compounds can be converted *in vitro* into the optivally inactive η-pyrromycinone (CXXIX) by heating *in vacuo* alone (ε), or in the presence of palladized charcoal (ζ). ε-Pyrromycinone is the common aglycon of pyrromycin, the cinerubins, and the rutilantins (*166*), which thus differ only in their carbohydrate components. Ettlinger *et al.* (*165*) have found that the ratio of cinerubins A and B, produced simultaneously by several species, is influenced by the culture medium; a soybean nutrient solution containing mannitol yields an excess of cinerubin A, but when glycerol replaces mannitol as the carbon source, cinerubin B preponderates. (The rhodomycinone and pyrromycinone pigments were obtained from cultures on synthetic media containing glycine and glycerol.) A feature of the glycosides in this group is the occurrence of unusual dimethyl-

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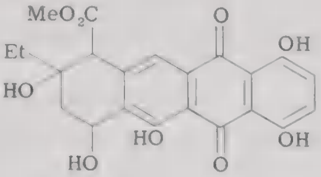
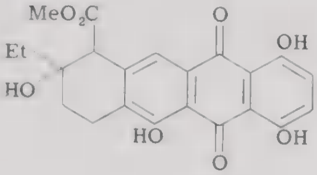
[*] Aklavin is another member of this series. The orange aglycone, aklavinone, has the same structure as ε-pyrromycinone less one of the phenolic groups (*166a*).

Naphthacenequinones



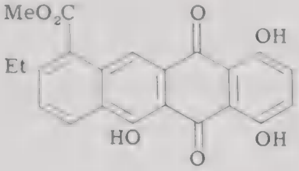
ε-Pyrromycinone
(Rutilantinone)

(CXXVII)



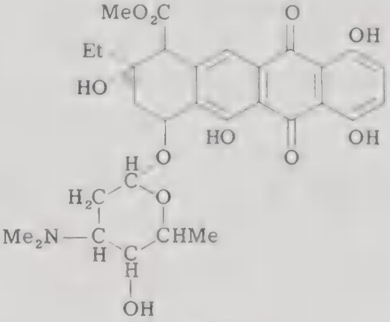
ζ-Pyrromycinone

(CXXVIII)



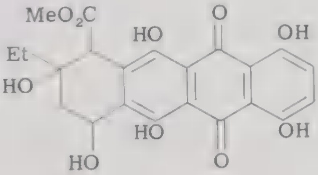
η-Pyrromycinone

(CXXIX)

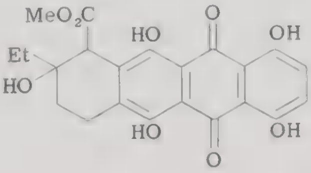


Pyrromycin

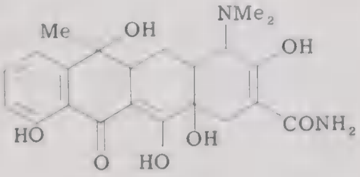
(CXXX)



ε-Isorhodomycinone



ζ-Isorhodomycinone



Terramycin

(CXXXI)

aminosugars, peculiar to bacteria. (A dimethylamino group is also present in the tetracyclines.) Thus pyrromycin (CXXX) is an ε-pyrromycinone rhodosaminide, and rhodomycin A is a combination of two molecules of β-rhodomycinone with three of rhodosamine. The cinerubins both yield three sugars on hydrolysis; two from each have the same R_f value and are probably identical, the third being different, and one of the trio in each case is a dimethylaminosugar of the rhodosamine or desosamine type. The rutilantins also contain aminosugars, not yet identified.

In their general survey of the natural distribution of coenzymes Q, Lester and Crane (*83*) detected these quinones in a number of bacteria. Coenzyme Q_8 has been isolated in a pure state from *Azotobacter vinelandii* (*115–117*), which produces comparatively large amounts, concentrated (6.0 μmoles per gram) in an electron transport particle. Identification in other cases is mainly dependent upon ultraviolet absorption spectra and behavior in reversed phase paper chromatography. Coenzyme Q_8 was thus detected in a *Hydrogenomonas* sp. and in cultures of *Escherichia coli*, but only when grown under aerobic conditions. In several other aerobic species (*Streptomyces griseus*, *Bacillus mesentericus*, and two *Mycobacterium* spp.) coenzymes Q could not be detected, but vitamin K was present. It may be that the latter is the respiratory quinone in these species since Brodie, Weber *et al.* (*169*) have shown that vitamin K is involved in electron transport in *Mycobacterium phlei*. Coenzyme Q_8 is not the only representative of this group in bacteria. Q_9 is present in *Pseudomonas fluorescens*, and in considerable amount (4.3 μmoles per gram) in the photosynthetic bacterium *Rhodospirillum rubrum*. In a *Chromatium* sp. this is replaced by Q_7, and *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) appears to contain more than one coenzyme Q (*150*).

Vitamin K_2. Quite early in the study of vitamin K it was noted that the experimentally induced deficiency disease of chicks, characterized by bleeding tendencies and blood coagulation defects, could be overcome by a diet containing fishmeal or rice bran which had been subjected to bacterial action. Fishmeal that had been washed, and either dried immediately or ether extracted, was ineffective, but if kept wet for some time to permit bacterial spoilage, an antihemorrhagic factor developed (*170*). Antihemorrhagic activity was observed also in the droppings of vitamin K-deficient chicks, and moreover, chicks could sometimes recover spontaneously from a K-deficient state (*171*). Later investigations showed that normal human adults excrete more vitamin K than their diet contains, the excess being formed by bacterial synthesis in the intestinal tract (*172*). After these observations, Almquist and coworkers (*173*) examined about twenty species of bacteria (including an organism isolated from putrefied fishmeal) in laboratory culture, and found that the great majority were able to synthesize vitamin K, as measured by the blood-clotting times of test chicks (see Table IV). Crane and Lester (*83*) have recently detected vitamin K in some other

TABLE IV

BACTERIAL SOURCES OF VITAMIN K[a]

Species	Method of identification[b]	Compound	References
Azotobacter vinelandii	UV	—	*(83)*
Bacillus brevis	P	$K_{2(35)}$	*(181)*
B. cereus	CT	—	*(173)*
B. mesentericus	UV	—	*(83)*
B. mycoides	CT	—	*(173)*
B. subtilis	CT	—	*(173)*
B. vulgatus	CT	—	*(173)*
Bacterium aerogenes	CT	—	*(173)*
B. flexneri	CT	—	*(173)*
B. friedlanderi	CT	—	*(173)*
B. proteus	CT	—	*(173)*
Chromatium sp.	UV	—	*(83)*
Erythrobacillus prodigiosus	CT	—	*(173)*
Escherichia coli	CT, UV	—	*(83, 173)*
Fishmeal	P	$K_{2(30)}$, $K_{2(35)}$	*(180, 183)*
Mycobacterium phlei	O	—	*(177, 185)*
M. tuberculosis	P	$K_{2(45)}$	*(184)*
M. smegmatis	UV	—	*(83)*
Salmonella typhosa	CT	—	*(173)*
Sarcina lutea	CT	—	*(173)*
Staphylococcus aureus	CT	—	*(173)*
Streptomyces griseus	UV	—	*(83)*

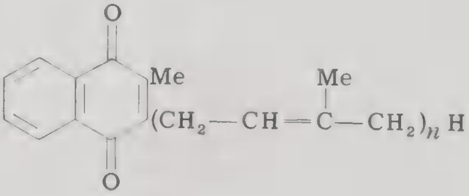
[a] More recent information will be found in reference *173a*.

[b] Abbreviations: CT, chick test; O, isolated as an oil; P, isolated in crystalline form; UV, ultraviolet absorption.

species by means of its characteristic ultraviolet absorption, before and after reduction with potassium borohydride.

One of the species studied by Almquist was *Mycobacterium tuberculosis*, from which Anderson and Newmann (*174*) had previously isolated phthiocol (3-hydroxy-2-methyl-1,4-naphthoquinone) by alkaline extraction of the acetone-soluble fraction of the lipids. This was found to have antihemorrhagic activity and because the first compound to be used successfully in vitamin K therapy (*175*). Phthiocol has also been obtained from *Corynebacterium diphtheriae* (*176*), but this, the first bacterial quinone to be positively identified, is almost certainly an artifact. Snow *et al.* (*177*) were unable to detect phthiocol in fresh extracts of *M. tuberculosis* but they did obtain a yellow oil having vitamin K properties, which gave phthiocol on alkaline hydrolysis. The possibility that phthiocol might arise from vitamin K under alkaline conditions had previously been suggested by Fieser *et al.* (*178*), who found that allylquinones in general react in this way, the allyl group being replaced by hydroxyl. Shortly afterward Fieser showed that phthiocol could be obtained from vitamin K_1 by treatment with alcoholic alkali (*179*). This in fact, occurs during the Dam-Karrer test (*49*) for vitamin K_1, the transient purple color changing to reddish brown as the anion of phthiocol is formed.

Crystalline vitamin K_2 was first isolated in 1939 by Doisy and his group from putrefied fishmeal (*180*). After extraction with light petroleum it was concentrated by chromatography and was finally crystallized in light-yellow plates, m.p. 54°. A few years later Tishler and Sampson (*181*) isolated the same vitamin from autolyzed cells of the soil bacterium *Bacillus brevis.* The structure was investigated by Doisy following the general procedure used in the study of vitamin K_1, in particular, ozonolysis of the leucodiacetate gave 1,4-diacetoxy-2-methyl-3-naphthaldehyde, previously obtained from vitamin K_1, together with acetone and levulinic aldehyde (nearly five moles). As these fragments accounted for forty-one carbon atoms, in agreement with the molecular formula $C_{41}H_{56}O_2$, Doisy *et al.* proposed structure (CXXXII; $n = 6$) for vitamin K_2 (*182*).



Vitamin K_2

(CXXXII)

No further chemical work was done in the vitamin K field for about ten years. In the meantime it had been observed that menadione (2-methyl-1,4-naphthoquinone) derivatives lacking a side chain at position 3, could not, in all cases, replace vitamin K_1 in vitamin K therapy, and this led to renewal of chemical and clinical investigation of the natural vitamins, especially by Isler and his collaborators in Basel. Using their immense experience in the synthesis of carotenoids they turned their attention to the polyisoprenoid naphthoquinones and succeeded in preparing a series of compounds with side chains containing two to seven isoprene units (CXXXII; $n = 2$ to 7) (*183*). A remarkable outcome of this work was the finding that Doisy's quinone, m.p. 54°, was not the difarnesyl derivative (CXXXII; $n = 6$) but 2-methyl-3-(all-*trans*)-farnesylgeranylgeranyl-1,4-naphthoquinone (CXXXII; $n = 7$) having a side chain of thirty-five carbon atoms. Furthermore, isolation of the natural product once more from putrefied fishmeal, revealed the presence of two K_2 vitamins, Doisy's compound, m.p. 54°, and also the lower isoprenolog, m.p. 50° (CXXXII; $n = 6$). These are now distinguished as vitamin $K_{2(35)}$ and $K_{2(30)}$, respectively.

Noll (*184*) has recently isolated a third crystalline vitamin of the K_2 series from *Mycobacterium tuberculosis* (Brévannes), the virulent human strain. This has been identified as vitamin $K_{2(45)}$, m.p. 60–61°, by comparison with synthetic material obtained by Isler and co-workers (*184a*). [The same compound, 2-methyl-3-solanesyl-1,4-naphthoquinone has also been synthesized by Folkers' group (*89*)]. Noll's vitamin is virtually identical with the yellow oil obtained by Snow *et al.* (*177*) from an unidentified strain of *M. tuberculosis* (and from *M. phlei*), and also with the yellow oil isolated from *M. phlei* by Brodie, Davis, and Fieser (*185*). According to Noll (*186*) the infrared spectrum of the latter definitely assigns it to the K_2 series (not K_1 as originally suggested*). This material, according to Brodie *et al.*, is remarkably active in restoring oxidative phosphorylation in extracts of *M. phlei* deactivated by irradiation.

The only other quinone relevant to this section is tetrahydroxybenzoquinone (CXXXV) which was first observed as a purple discoloration on a sample of salted beans (*187*). Investigation revealed (*188*) that the pigment is formed by *Pseudomonas beijerincki* Hof from *myo*-inositol under rather special conditions (an alkaline medium contain 6–18% NaCl and low oxygen tension), the purple color being that of the calcium (and perhaps also the magnesium) salt. (The corresponding salts of rhodizonic acid may also be present.) This has recently been confirmed by Weygand *et al.* (*189*) using labeled *myo*-inositol as substrate. In agar cultures, some of the pigment appears at a considerable distance from the bacterial colonies, the characteristic distribution strongly indicating that a colorless intermediate is formed initially by the bacterium, which is subsequently autoxidized. Since *Acetobacter suboxydans* Kluyver and de Leeuw can dehydrogenate *myo*-inositol to a monoketone, Kluyver *et al.* (*188*) suggested that *P. beijerincki* might similarly convert *myo*-inositol into a triketone (CXXXIII) which, in alkaline solution, would enolize to hexahydroxybenzene (CXXXIV) and then rapidly oxidize to tetrahydroxybenzoquinone (CXXXV). If this is so, the steric requirements of *P. beijerincki* are less severe than those of *Acetobacter suboxydans* which will dehydrogenate only axial hydroxyl groups (*190*). The Dutch workers found that *myo*-inositol could be replaced by L-inositol, but not by quercitol, quebrachitol, pinitol, or quinic acid, nor could *P. beijerincki* be replaced by other *Pseudomonas* spp.

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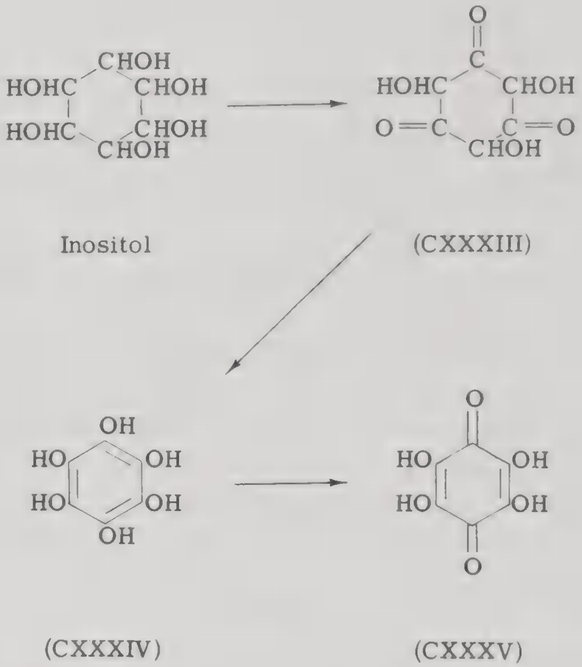
* However, *M. phlei* produces more than one quinone (*56*).



Inositol

(CXXXIII)

(CXXXIV)

(CXXXV)

Since *myo*-inositol occurs widely in nature, its conversion into an aromatic compound is of considerable biogenetic interest (*189*).

B. The Animal Kingdom

1. *Protozoa*

There are only two reports of quinones in this phylum. A coenzyme Q has been detected in *Tetrahymena pyriformis* (*150*), and Lederer and co-workers (*191*) have isolated two pigments from the ciliate catharobe *Stentor niger* Ehrb. that behave like hydroxyquinones in several respects, although they do not appear to be easily reducible. The principal red-violet coloring matter stentorol shows a resemblance to hypericin and the erythroaphins, but little is known about the second brown pigment.

2. *Annelida*

Although pigments abound in these animals, few have been examined, and only two of these show definite quinone-like character. One is the compound arenicochrome, present as green granules in the epithelial cells of the lugworm *Arenicola marina* L. It can be isolated by alkaline extraction and crystallized as its blue tripotassium salt $(C_{21}H_{15}O_{14}K_3)_n$ ($n = 1$ or 2). Van Duijn *et al.* (*192*) obtained 50 mg. from about a thousand worms. Arenicochrome is a disulfuric ester, and on treatment with acid at room temperature, sulfuric acid is split off leaving a violet pigment, arenicochromine, $C_{42}H_{34}O_{16}$ (or perhaps $C_{40}H_{32}O_{15}$). Arenicochromine shows two color changes in solution, one from red to green at about pH 6, and another from green to purple at about pH 9. It can be reduced with alkaline sodium dithionite, forming a yellow solution which returns to green on shaking in air. It appears to be a polyhydroxypolycyclicquinone.

The second pigment is hallachrome (*192a*), an acidic red coloring matter present in the integument of the polychete worm *Halla parthenopeia* (Della Chiaje) originally thought to have the structure, 2,3-dihydroindole-5,6-quinone-2-carboxylic acid. It gives a green solution in alkali, changing reversibly to blue violet with acid, and is readily reduced to a yellow leuco compound, the original color being restored in air. Beyond this, it is doubtful if any of the original work is reliable, but recent work by Bielig and Möllinger (*192b*) suggests that hallachrome, $C_{21}H_{24-25}NO_9S$, may be a hydroxyquinone sulfonic acid. A closely related vermilion pigment, hallorange, is also present.

3. *Arthropoda*

In this phylum a group of simple benzoquinones has been identified in Myriapoda, Insecta, and Arachnida. In the order Hemiptera (Insecta), a few Coccidae secrete anthraquinones, and complex aphin pigments (extended quinones) are found in a number of Aphididae. Once again there is great diversity of structure, and as the number of species examined is relatively trivial, little correlation can be attempted. No quinones have yet been found in the Crustacea. This section deals only with quinones that have been isolated as such and is not concerned with the intermediate formation of quinones involved in the hardening of the cuticle of arthropods. This subject has been reviewed recently (*193*).

a. Benzoquinones. The simplest of all quinones are found in insects and millepedes. The discharge of obnoxious gases by certain insects has been well known for a long time, but only in the last few years, through the application of modern techniques, has the presence of quinones been definitely established, mainly by the work of Roth, Lederer, Pavan, and Schildknecht. Many observers have noted that millepede secretions are yellow or brown and have an odor likened to that of the halogens or nitrogen peroxide (*194*). Such exudates probably contain benzoquinones. *p*-Benzoquinone itself has been positively identified in the beetle *Brachinus crepitans* (Linn.) (*195*), in the cockroach *Diploptera punctata* (Eschscholtz) (*196*), and in the millepede *Spirostreptus castaneus* Attems (*197*). Earlier claims that benzoquinone is also emitted by the millepede *Julus terrestris* (*198*) and the cockroach *Blaps gigas* Fischer (*199*) were not supported by adequate evidence. Nevertheless it is fairly certain that a simple quinone (or quinones) does occur in those species. Reference to Table V shows that methylbenzoquinone (toluquinone) occurs most frequently, both in Insecta

TABLE V

DISTRIBUTION OF BENZOQUINONES IN ARTHROPODA[a,b,c]

Species	Quinones	References
Insecta, Coleoptera		
Brachinus crepitans (Linn.)	Benzoquinone, methylbenzoquinone	(*195*)
Tenebrio molitor Linn.	Methylbenzoquinone	(*206*)
Tribolium confusum J. du V.	Ethylbenzoquinone, methylbenzoquinone	(*211*)
Tribolium castaneum Herbst.	Ethylbenzoquinone, methylbenzoquinone, and methoxybenzoquinone	(*208*)
Diaperis boleti (Linn.)	Ethylbenzoquinone, methylbenzoquinone	(*215a*)
Diaperis maculata Olivier	Ethylbenzoquinone, methylbenzoquinone	(*196*)
Blaps gigas Fischer	Benzoquinone?	(*199*)
Blaps mucronata Latr.	Ethylbenzoquinone, methylbenzoquinone	(*215*)
Blaps requieni Solier	Ethylbenzoquinone, methylbenzoquinone	(*215*)
Insecta, Dictyoptera		
Diploptera punctata (Esch.)	Benzoquinone, ethylbenzoquinone, and methylbenzoquinone	(*196*)
Myriapoda, Diplopoda		
Julus terrestris	Benzoquinone?	(*198*)
Spirostreptus castaneus Attems	Benzoquinone	(*197*)
S. multisulcatus Demange	Methylbenzoquinone	(*207*)
S. virgator Silvestri	Methylbenzoquinone	(*197*)
Archiulus sabulosus L.	Methylbenzoquinone, 2-methyl-3-methoxybenzoquinone	(*212*)
Pachybolus laminatus Cook	Methylbenzoquinone	(*197*)
Aulonopygus aculeatus Attems	Methylbenzoquinone	(*207*)
A. aculeatus barbieri Demange	Methylbenzoquinone	(*207*)
Peridontopyge vachoni Demange	Methylbenzoquinone	(*207*)
P. aberrans Attems	Methylbenzoquinone	(*207*)
Arachnida, Opiliones		
Gonyleptidae sp.	2,3-, 2,5-Dimethylbenzoquinone, 2,3,5-trimethylbenzoquinone	(*201*)

[a] Additions to this list are given in references *215* and *215a*.

[b] Unidentified quinones have been detected in the following species: *Tribolium destructor* Uytt (*208*), *Blaps gibba* Linn. (*213*), *Latheticus oryzae* Wat. (*208*), *Scotobates calcaratus* (Fabr.) (*214*), *Uloma impressa* Melsh (*214*), and *Spirostreptus virgator* Silvestri (*197*).

[c] Ubiquinones are not included in this list.

and Myriapoda, sometimes along with other quinones. The most notable mixture is the material gonyleptidine, a yellow, aqueous, antibiotic fluid, containing 2,3- and 2,5-dimethyl-, and 2,3,5-trimethylbenzoquinone, secreted by a South American arachnid of the Gonyleptidae family. The antibiotic nature of the secretion from *Tribolium confusum* and *T. castaneum* had been noted (*200*) previously, and the examination of gonyleptidine by Fieser *et al.* (*201*) revealed *inter alia* that simple alkylbenzoquinones are more potent than any other type of quinone (cf. *202*).

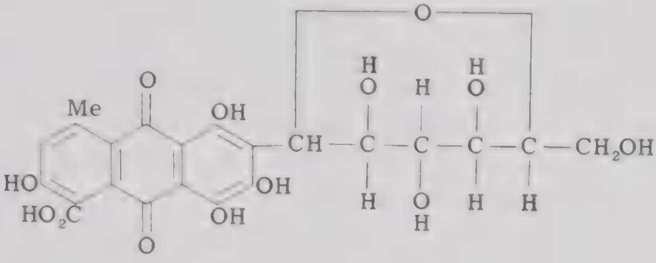
It appears that the function of the simple quinones listed in Table V is to provide a means of defense for these animals (*202a*). They are discharged when the animals are disturbed, and advantage is taken of this behavior in laboratory procedures for their detection and isolation. Roth (*203*) showed that a blue color could be obtained on acidified starch iodide paper merely by holding a beetle (e.g., *Tribolium confusum*) over it. Tenebrionids generally respond to this simple test, although a negative reaction does not necessarily mean that odoriferous glands are absent, and a positive test is not specific for quinones (as distinct from other oxidizing agents) (*204*). The most remarkable example is the bombardier beetle *Brachinus crepitans* (= *explodens*), which discharges its quinones explosively in an acrid cloud when irritated. The secretion [pH 4.2, but *not* nitric acid, as first suggested (*205*)] is a mixture of benzoquinone and toluquinone in a fatty oil (with occasional traces of uric acid), which is ejected together with a gas consisting mostly of oxygen, with lesser amounts of carbon dioxide and nitrogen (*195*). Schildknecht (*206*) and Barbier (*207*) have recently devised techniques whereby the quinones discharged from a single animal can be collected and identified by chromatography. On a larger scale, the odorous secretion from batches of insects can be isolated by direct vacuum sublimation. In this way Loconti and Roth (*208*) obtained 30 gm. of mixed quinones from over 1.5 million flour beetles (*Tribolium castaneum*).

The odoriferous glands of a number of beetles (Tenebrionidae) (*203, 204*) and of the cockroach *Diploptera punctata* (*196*) have been studied in detail by Roth. Infrared analysis of the secretion from the latter showed that the relative concentrations were methylbenzoquinone > ethylbenzoquinone > benzoquinone, males having a slightly higher proportion of methylbenzoquinone than females, but in the last and next-to-last instar nymphs of both series, the order of relative concentrations was ethylbenzoquinone > methylbenzoquinone > benzoquinone. *D. punctata* differs from the tenebrionids in that immature stages, as well as the adults, produce quinones. In both the cockroach and the beetles, quinone biosynthesis develops rapidly after emergence of the adult, and there is a progressive increase with age of the quantity of yellow fluid in the gland sacs.

The biogenesis of these quinones is obscure. Brunet and Kent (*209*, *210*) have shown that an *o*-quinone produced by the colleterial glands of the cockroach *Periplaneta americana* is formed from the β-glucoside of a phenolic precursor, protocatechuic acid. Homogentisic acid, which could be derived from tyrosine, is a possible phenolic precursor, and so of course is quinol, which, according to Dennell (*193*), is formed in the larval cuticle of *Calliphora vomitoria* from phenylalanine and tyrosine and is utilized there for quinone tanning. The hydroxylating system which gives rise to quinol is nonenzymatic and nonspecific, and is possibly not confined to the cuticle. However, although Roth and Stay (*196*) found both a β-glucosidase and a polyphenoloxidase in the tracheal glands of *D. punctata*, no β-glucoside or other phenolic precursor could be detected. Rao and Seshadri (*202*) have drawn attention to an interesting relationship between these quinones and the tocopherols. The latter can be synthesized *in vitro* by condensing quinols with phytol, and the *in vivo* synthesis may be essentially similar. It is not known if this occurs in animals, but it is noteworthy that the quinols corresponding to the alkylated quinones in Table V could, if condensed with phytol, provide all the known tocopherols.

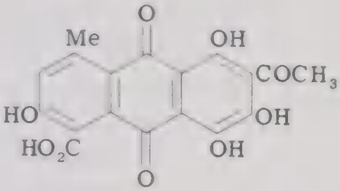
Ubiquinones also appear in insects. Crane and Lester (*83*) identified coenzyme Q_9 in the housefly (*Musca domestica*) and the cabbage butterfly (*Pieris rapae*) by extraction of the whole organism, and Morton (*216*) has found both Q_9 and Q_{10} in the larvae of the blowfly. Heller and Szarkowska (*217*) have demonstrated the presence of quinone reductase in the bodies of the pupa and imago of the hawk moth *Celerio euphorbiae* Linn. and concluded that a quinone was involved in the respiratory system of these animals. No quinone was identified, but it seems likely that ubiquinones play an integral part in insect respiration.

b. Anthraquinones. Three of the most important natural dyestuffs are closely related anthraquinones produced by Coccidae. These are the following: (*a*) Carminic acid (CXXXVI) (*218*), the coloring principle of cochineal, which consists of the dried bodies of the Mexican species *Dactylopius coccus* Costa, found on various Cactaceae. The same, or a closely related pigment, is produced by the South American species *D. confusus* Cockerell, *D. tomentosus* Lamarck, and *D. indicus* Green, and probably also by species of the European genus *Porphyrophora*, e.g., *P. polonicus* Linn. Cochineal still enjoys a limited use, mainly in the coloring of food. (*b*) Kermesic acid (CXXXVII) is the principal pigment of kermes (*219*), probably the most ancient dyestuff on record. This was formerly obtained from *Kermococcus ilicis* Linn., which infests the kermes oak (*Quercus coccifera* Linn.) (*c*) Laccaic acid (*220*) is the



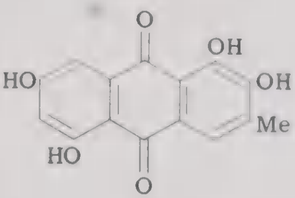
Carminic acid

(CXXXVI)



Kermesic acid

(CXXXVII)



Erythrolaccin

(CXXXVIII)

red coloring matter of lac dye, a crude extract obtained from lac. The latter is a mixture of resinous substances, wax, sugars, and pigment secreted by various coccids, in particular by *Laccifer lacca* Kerr, which lives on the twigs of various host trees (e.g., *Butea frondosa* Roxb.) in India and southeastern Asia. Shellac is still manufactured from lac, but the dye is no longer of commercial interest. Laccaic acid is water soluble, like carminic acid, and is probably similar in structure to the other two red pigments, but it appears to be a mixture (*221*). There is also present in lac a yellow, water-insoluble quinone, erythrolaccin (*222*), to which Venkataraman (*223*) has recently allotted structure (CXXXVIII). Carminic acid and kermesic acid are more complex than the polyhydroxyanthraquinones found in the plant kingdom and differ from the latter in possessing carbon side chains in an α-position. Carminic acid belongs to the (at present) small group of naturally occurring *C*-glycosyl compounds. Such compounds may be numerous, but as they are resistant to normal acid hydrolysis they are not detected when searching for *O*-glycosides by normal methods.

Although these pigments have been of great value to man, they have no obvious biological significance for the insects themselves, and, like the aphins, they seem to be merely by-products of metabolism. Sap-sucking insects ingest large quantities of plant juice, mostly carbohydrate in content, and the quinones most probably arise from this, perhaps through the metabolic activity of symbiotic microorganisms in the mycetomes (*224*). The flesh of many Cactaceae has been observed to redden when cut and exposed to air, and two of the host trees of the lac insects *Butea frondosa* (Leguminosae) and *Zizyphus jujuba* (Rhamnaceae)[*] belong to families which elaborate anthraquinones, but there is no evidence at present that suggests that the insect pigments or substantial precursors thereof are obtained directly from the plants on which they feed.

c. Aphins. These remarkable pigments were first examined ninety years ago by Sorby (*225*) in a study of the "red Aphides" found on the bark of apple trees [presumably the common woolly aphid *Eriosoma lanigerum* (Hausmann)]. Isolated observations were recorded later by other workers but the structures of these compounds remained unknown until recently. The researches of Todd and his collaborators have shown that the aphins are complex polycyclic quinones built on a coronene framework. The hemolymph of the living insect contains a yellow, water-soluble pigment, protoaphin, which is converted by enzymatic action after death, into a yellow fat-soluble fluorescent xanthoaphin. In crude extracts the xanthoaphin changes rapidly into an orange chrysoaphin and then more slowly into a red erythroaphin, the stable end product (*226, 227*). These changes take place rapidly in the presence of acids or bases, or on heating, but the protoaphin is relatively more stable, especially to heat. If aphids are killed by rapid heating to 70° enzymes are simultaneously inactivated, but the protoaphin survives and can be extracted with aqueous methanol. The pigments isolated from *Aphis fabae* are shown below.

$$\begin{array}{c} \text{Protoaphin-}fb \\ C_{36}H_{40}O_{16} \\ \text{Yellow,} \\ \text{water-soluble,} \\ \text{nonfluorescent} \end{array} \longrightarrow \underbrace{\begin{array}{ccccc} \text{Xanthoaphin-}fb & \longrightarrow & \text{Chrysoaphin-}fb & \longrightarrow & \text{Erythroaphin-}fb \\ C_{30}H_{26}O_{10} & & C_{30}H_{24}O_{9} & & C_{30}H_{22}O_{8} \\ \text{Yellow} & & \text{Orange} & & \text{Red} \end{array}}_{\text{fat-soluble, fluorescent}}$$

Aphins have been detected in some forty species of aphids (*228*). About half of them are listed in Table VI; the remainder have not been identified with certainty and are omitted. (In a further twenty species aphins were not detected.) It will be noted (Table VI) that these pigments are not confined to the Aphididae; indeed one of the first pigments of this type to be isolated, strobinin, was obtained by Blount (*229*) from *Adelges* (*Pineus*) *strobi* of the family Phylleroxidae. In most of the species listed the interrelationship between all four types of pigment has been established, and erythroaphins from seven species have been isolated in crystalline form. Most work has been done on *Aphis fabae* (infesting broad beans) and *Tuberolachnus salignus* (parasitic on willow), which were fairly readily accessible and contain appreciable amounts of pigment ($<2\%$ of the live weight in *T. salignus*); from each of these, all four pigments have been isolated. Not all species produce the same pigments, and different aphins were initially designated by a suffix indicative of the species of origin, e.g., protoaphin-*fb* from *A. fabae*. Different erythroaphins are not easily distinguished, and it is necessary to resort to comparison of infrared spectra and X-ray diffraction photographs. Of the erythroaphins isolated later, four were found to be identical with erythroaphin-*fb* (see Table VI) and erythroaphins-*fb* and -*sl* proved to be stereoisomers. On this system of nomenclature, Blount's strobinin is erythroaphin-*st;* lanigerin, which he iso-

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[*] Maurin (*224a*) claims to have detected anthraquinones in the bark of *Z. vulgaris.*

TABLE VI

DISTRIBUTION OF APHIN PIGMENTS

Species	Erythroaphin[a]		References
	Detected	Isolated	
Aphididae			
Aphis fabae Scop.	...	*fb*	(*226*)
A. rumicis L.	+	...	(*228*)
A. philadelphi (Börner)	+	...	(*228*)
A. viburni Scop.	+	...	(*228*)
A. cognatella (M. G. Jones)	+	...	(*228*)
A. hederae Kalt.	+	...	(*228*)
A. sambuci L.	...	*fb*	(*228, 239*)
A. ilicis Kalt.	+	...	(*228*)
Brachycaudus klugkisti (Börner)	+	...	(*228*)
B. rociadae	+	...	(*228*)
Dactynotus (*Uromelan*) *jaceae* (L.)	+	...	(*228*)
Eriosoma lanigerum (Hausmann)	...	*fb*	(*229, 239*)
E. ulmi (L.)	+	...	(*228*)
Myzus cerasi (F.)	...	*fb*	(*228, 239*)
Rhopalosiphum nymphacae (L.)	+	...	(*228*)
Sappaphis pyri (Fonsc.)	...	*py*	(*228*)
Schizolachnus tomentosis (de Geer)	+	...	(*228*)
Tuberolachnus salignus (Gmelin)	...	*sl*	(*227*)
Adelgidae			
Adelges (*Pineus*) *strobi* (Börner)	...	*st*	(*228, 229, 239*)
Adelgid (unidentified)	+	...	(*228*)

[a] Note that erythroaphins are artifacts.

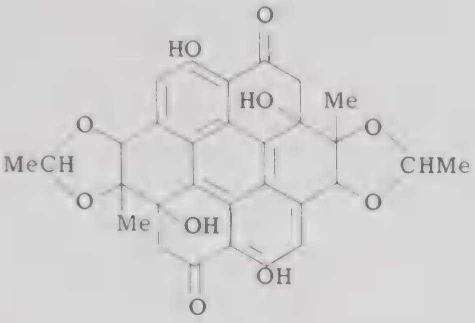
lated from *Eriosoma lanigerum*, has been re-examined and shown to be a chrysoaphin readily converted into erythroaphin-*fb*.

The erythroaphins have the chromophoric system of 4,9-dihydroxyperylene-3,10-quinone (CXLII) with additional saturated rings fused to each side (*230*). The arrangement of the latter is not completely established, but the most recent work (*231*) favors structure (CXLI) for erythroaphins-*fb* and *sl*. In erythroaphin-*fb* both dioxolan rings are *cis*-fused to the rest of molecule, whereas in erythroaphin-*sl* one of these rings has the less stable *trans*-fusion (*231, 232*). It is of interest that 4,9-dihydroxyperylene-3,10-quinone itself, a dark red, almost black pigment, is produced by the large ascomycete *Daldinia concentrica* (Bolt.) Ces and de Not. (*233*), and several species of fungi of the phytopathogenic genus *Elsinöe* (Ascomycetes) and of its conidial stage *Sphaceloma*, produce red fluorescent pigments which are spectroscopically very similar to the erythroaphins (*234*). Cercosporin, $C_{30}H_{28}O_{10}$, a deep-red metabolic product of the fungus *Cercosporina kikuchii* M. and T., appears to be of the same type (*235*). It must be borne in mind however, that the erythroaphins are artifacts.

It will be noted from the scheme that the conversion of xanthoaphin into chrysoaphin and then into erythroaphin, involves the loss of one molecule of water at each stage and the disappearance of hydroxyl groups (as shown by the infrared spectra). The xanthoaphins and chrysoaphins are now considered (*231*) to have structures (CXXXIX) and (CXL), respectively, reminiscent of flavoskyrin (CLXIX) and rubroskyrin (CXVI), and their transformation into (CXLI) effects a progressive aromatization of the molecule. Xanthoaphin-*sl* is a mixture of two, and chrysoaphin-*sl* of three, stereoisomers of undetermined configuration, all of which can be converted into the unique erythroaphin-*sl*. The latter can be isomerized to erythroaphin-*fb* by treatment with alkali (*236*).

The structures of the protoaphins, which exist in the living insects, have not yet been elucidated. Acid hydrolysis of protaphin-*fb* gives D-glucose, small amounts of erythroaphin, and considerable amounts of unidentified materials (*237*). Protoaphin-*fb* thus appears to be a mono-*O*-glucoside. Enzymatic conversion of protoaphin into xanthoaphin is much more efficient, but the reaction is not a simple hydrolysis since one atom of oxygen per molecule is taken up from the air in the process (*231*). Enzymes from one species of aphid can effect the protoaphin → xanthoaphin transformation in another species, e.g., protoaphin-*ph* (from *A. philadelphi*) can be converted into xanthoaphin-*ph* at pH 6.5 by small amounts of freshly pulped *A. sambuci* or T. *salignus*. On the other hand no conversion takes place on adding species containing no protoaphin, e.g., *Hamamelistes betulae*, indicating that the enzyme(s) concerned occur only in those insects that contain protoaphin.

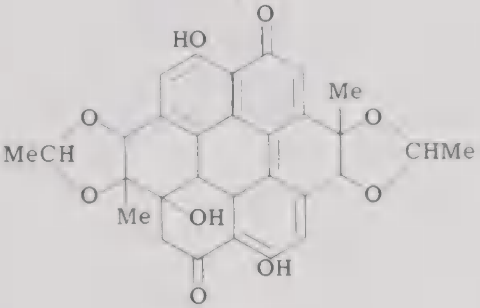
A related group of aphin pigments occurs in the primitive aphids



Xanthoaphin

(CXXXIX)

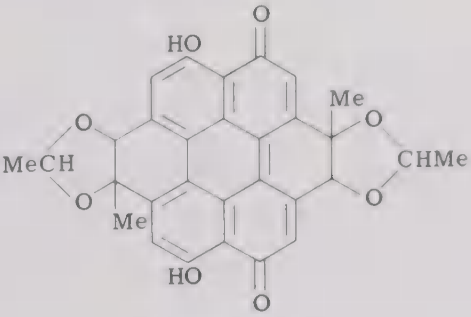
$\downarrow -H_2O$



Chrysoaphin

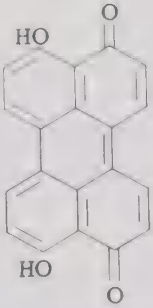
(CXL)

$\swarrow -H_2O$



Erythroaphin

(CXLI)



(CXLII)

Hamamelistes spinosus Shimer and *H. betulae* Mordvilko (*238*). Red, water-soluble pigments, heteroaphins, are present in the hemolymph (approximately 1% of the live-insect weight), which are converted by enzymes (or acid) into a red, ether-soluble rhodoaphin, $C_{30}H_{22}O_{11}$. This can be reduced with zinc and acetic acid to an erythroaphin, and it appears on present evidence, to be a hydroxylated erythroaphin.

4. *Echinodermata*

Quinone pigments make their only real contribution to animal coloring in this phylum. The varied hues of the spines and test (shell) of many echinoids are due to the presence of polyhydroxynaphthoquinones. A similar pigment occurs in at least one holothuroid, and polyhydroxyanthraquinones have been found recently in crinoids.

a. Echinoidea. A red pigment, "echinochrome," present in the coelomocytes of *Paracentrotus lividus* (Lam.) and *Echinus esculentus* Linn., was first described by MacMunn (*240*). The pigment was later isolated from the eggs, ovaries, and other parts of *Arbacia lixula* (Linn.) by several workers and was named echinochrome A by Kuhn and Wallenfels (*241*), who determined its structure (CXLIII). Comparatively large amounts of echinochrome A are present in the ovaries of this species—as much as 10 mg. in a single ovary—but there is a seasonal variation. Kuhn and Wallenfels (*242*) found that echinochrome A was abundant in mature ovaries in March and April, but in the following November and January the immature ovaries, which are small and deep violet in color, contained relatively little echinochrome A but small amounts of two new pigments, echinochromes B and C, had appeared. Most of these latter had disappeared by the following March and much echinochrome A was again present in the red-brown ovaries. Echinochrome B is a red compound, m.p. 173–175°, which appears to be similar in properties to echinochrome A. Only very small amounts of the violet echinochrome C were obtained. Spectroscopically it was similar to echinochromes A and B but could not be extracted from ether with aqueous sodium hydroxide. Glaser and Lederer (*243*) also found a high concentration of echinochrome A in the ripe ovaries of *A. lixula* but ripe testes contained none. On the other hand echinochrome A, combined with protein, is present in the blood cells of both male and female animals (*244*). Echinochrome-like pigments have been detected spectroscopically in the organs of other species but are not invariably present; e.g., two such compounds occur in the ectoderm, endoderm, intestine, ovary, spermary, and egg cases of the purple sand dollar, *Dendraster excentricus*, whereas *D. laevis* contains none (*245*, *246*). A similar pigment is present in the pale urchin *Lytechinus pictus*; the amount is small, but aggregates are observable in the gonads of both sexes and in other tissues (*245*).

The quinones present in the spines and test of sea urchins, in addition to echinochrome A which is also a fairly common spine pigment, are known as spinochromes (see Table VII). They are closely related to echinochrome A in structure but so far have been found only in calcareous parts of the animals. However most workers interested in the isolation of these pigments have examined only the spines and test, and the occurrence of spinochromes in other organs is not excluded. In a recent examination of the tropical sea urchin *Diadema antillarum* Philippi, Millott (*247*) has shown that the pigment (probably echinochrome A) is widely distributed throughout the body of the animal including the calcified regions, and this may be true of other species. Pigments present in the soft parts of these animals appear red or purple, but the spines and tests may be green, olive, brown, or purple, the colors being those of the calcium salts of the hydroxynaphthoquinones. (Not all species are colored, and some are black when melanin is present.) The pigments themselves, all of which are red, can be ex-

TABLE VII
DISTRIBUTION OF SPINOCHROMES AND ECHINOCHROME A IN THE SPINES AND TESTS OF ECHINOIDEA[a]

Species	Spinochrome[b]										Echinochrome	References
	A	B	C	D	E	F	G	M	N	P	A	
Anthocidaris crassisipina								+	+			(*267, 268*)
Arbacia lixula			+								+	(*250, 269*)
Diadema antillarum											+[c]	(*247*)
Diadema setosum											+	(*270*)
Echinus esculentus	+	+									+	(*248, 249*)
Hemicentrotus pulcherrimus									+			(*271*)
Heterocentrotus mammilatus						+						(*267*)
Paracentrotus lividus (North Atlantic)	+	+										(*243, 249*)
Paracentrotus lividus (Mediterranean)		+	+		+		+			+	+	(*250, 272*)
Psammechinus miliaris	+[c]	+[c]			+							(*273*)
Pseudocentrotus depressus				+								(*267*)
Scaphechinus mirabilis											+	(*274*)
Strongylocentrotus purpuratus											+	(*256*)

[a] Nomenclature according to Th. Mortensen "A Monograph of the Echinoidea," Vols. 1–5. Reitzel, Copenhagen, 1929–1951.

[b] For nomenclature see references *1* and *275*.

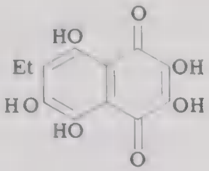
[c] Not definitely identified.

tracted by treating the spines with hydrochloric acid under ether. If the ether solution is then chromatographed on a column of calcium carbonate, the original colors of the spines are reproduced on the column. It will be noted in Table VII that the spinochromes frequently occur as mixtures, a different ratio producing color variations within the same species. Thus Goodwin and Srisukh (*249*) have found the amounts of spinochrome A and B in violet and olive-green specimens of *Paracentrotus lividus*, as shown in the tabulation. A third pigment, echinochrome A, is also present in the spines of these violet and green animals collected from North Atlantic waters (*250*), whereas brown specimens, obtained from the Mediterranean, contain a mixture of spinochromes B, C, E, G, and P. It is of interest that the bones of some sea otters (*Enhydra lutris*) are a bright purple color. Spectroscopically the pigment shows a close resemblance to echinochrome A, and it is known that these animals feed upon sea urchins (*251*).

Paracentrotus lividus	Spinochrome A (mg./gm. fresh wt.)	Spinochrome B (mg./gm. fresh wt.)
Violet spines	0.52	0.39
Olive green spines	0.17	1.07

The structures allotted to the spinochromes are shown in formulas (CXLIV) to (CLI). Some of these (B, M, and P) require further confirmation, and only the structure of spinochrome N has been established by laboratory synthesis (*252*). [Echinochrome A has also been synthesized (*253*)]. Similar compounds, in a lower state of oxidation, are found in plants and fungi; e.g., spinochrome D is a dihydroxy derivative of hydroxydroserone (L) and spinochrome N is a monohydroxy derivative of flaviolin (XCIV). The origin of these pigments is obscure. Animal biosynthesis of the naphthalene system is still uncertain, and these quinones, or substantial precursors thereof, may come from their diet. Some sea urchins are mainly herbivorous and others are chiefly carnivorous; both groups produce naphthoquinones, but to date no compounds of this type have been found in other forms of marine life (excepting related animals, see below). This suggests that the echinoid quinones are endogenous, but it is well to remember that echinenone once appeared to be the carotenoid peculiar to sea urchins but was later found in algae; here too, the origin of the animal pigment is in doubt.

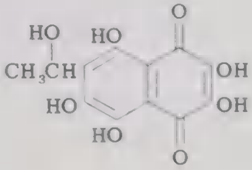
Sea urchins are peculiar animals in many ways. The presence of naphthoquinones is one of their peculiarities, and the wide distribution of these pigments in the body (of certain species at least) must be of some significance. In *Echinus esculentus* (and other species) part of



Echinochrome A

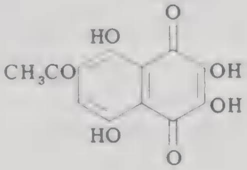
(CXLIII)

Spinochromes[a]



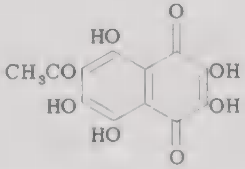
Spinochrome A

(CXLIV)



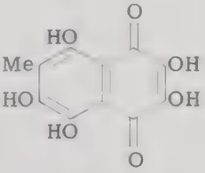
Spinochrome B

(CXLV)



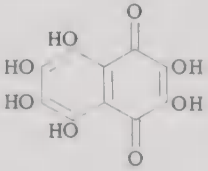
Spinochrome C

(CXLVI)



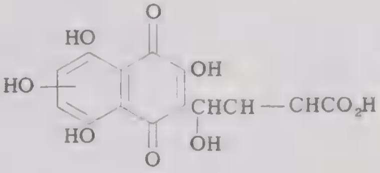
Spinochrome D

(CXLVII)



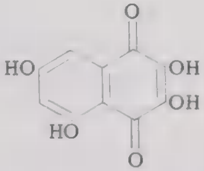
Spinochrome E[b]

(CXLVIII)



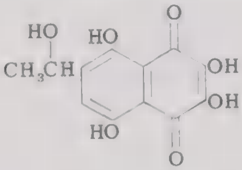
Spinochrome M

(CIL)



Spinochrome N

(CL)



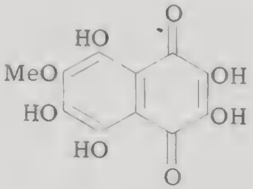
Spinochrome P

(CLI)

[a] For references to individual species see (*1*).
[b] See reference *273*.

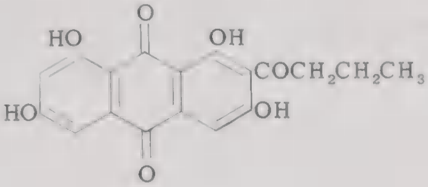
the pigment occurs in a reduced form in the perivisceral fluid, but an early suggestion that it had a respiratory function could not be confirmed (*254*). Most interest has centered on the pigments present in the eggs and sex organs. Friedheim (*255*) alleged that the presence of echinochrome A caused a sixteenfold increase in the respiratory rate of unfertilized eggs (*P. lividus*), but this was refuted by Tyler (*256*). It has long been known that sea urchin eggs secrete substances that stimulate sperm in a powerful manner. In some species this secretion is a protein complex containing echinochrome A, and according to Hartmann *et al.* (*257*) the pigment itself (from the eggs of *Arbacia lixula*) has the same activating properties and is effective in a dilution of one part in 2.5×10^9 parts of sea water. This has been denied by other workers (*256, 258*), and Cornman (*258*) has pointed out that echinochrome A is of limited occurrence in the eggs of Echinoidea, and moreover egg secretions from pigmented eggs will stimulate sperm from unpigmented species, and vice versa. The wider distribution of naphthoquinones in the body and spines of these animals may be of more significance. Recent work by Millott and Yoshida (*259*) on the spectral sensitivity of echinoids suggests the possibility that these pigments may be involved in photoreception. Sea urchins are highly sensitive to light of appropriate wavelength and it was found that the maximum sensitivity of *Diadema antillarum* (at 465 mμ) almost coincided with the absorption maximum (462–463 mμ) of the quinone pigment found throughout this animal. Further work in this direction may reveal the true function of these coloring matters.

b. Holothurioidea. There is only one record of the occurrence of a quinone in this class. Mukai (*260*) has recently found that the purple pigment of the sea cucumber *Polycheira rufescens* (Brandt) is a chromoprotein readily extracted from whole animals with aqueous acetic acid. After precipitation with methanol and treatment with hydrochloric acid, he obtained a crimson pigment, namakochrome, $C_{11}H_8O_8$, 218°. This has proved to be 7-methoxy-2,3,5,6,8-pentahydroxy-1,4-naphthoquinone (*260a*), i.e., spinochrome E monomethyl ether, and is the first example of a spinochrome occurring outside the Echinoidea.



Namakochrome

c. Crinoidea. Although the colors of crinoids are most beautiful and varied, they have attracted surprisingly little attention. There are early reports (e.g., *261*) on the pigments of *Antedon* spp., but no chemical investigation was attempted until very recently. Fossil pigments found in crinoid limestone by Blumer (*262*) may be complex polyhydroxy-quinones. Dimelow (*263*) has isolated a very small amount of a red pigment occurring in the eggs and connective tissue of the macrophreate species *Antedon bifida* (Pennant). It gives a violet solution in aqueous sodium bicarbonate, and spectroscopically it could be either a hydroxynaphthoquinone or a hydroxyanthraquinone, but its high acidity (p*K* approximately 3.8) favors the former (*264*). Sutherland and Wells (*265*) have recently obtained comparatively large quantities of pigment from the Australian oligophreate species *Comatula pectinata* Linn. (about 5% of the dry weight). Chromatography revealed a mixture of eight coloring matters, at least two of which are polyhydroxyanthraquinones, probably the 6-monomethyl ether and 1,6-dimethyl ether of rhodocomatulin (CLII). These are closely related in structure to the lichen pigment solorinic acid (CXIX). A similar mixture occurs in *C. cratera* H. L. Clark, at least three components being identical with those of *C. pectinata*, and hydroxyanthraquinones are also present in *Ptilometra australis* Wilton (*266*).



(CLII)

5. *Chordata*

The early investigations of Dam and others (*53*, *276*) showed that many animal tissues possessed vitamin K activity. Hog liver fat contained appreciable quantities of an active factor, smaller amounts were found in the liver of the dog, and traces in egg yolk, milk, etc. Recently the presence of vitamin K in beef liver has been demonstrated by chick assay (*277*), but direct chemical evidence for the existence of vitamin K compounds in animal tissues is still lacking, and until very recently no quinone had been isolated from higher animals. Martius and Esser (*278*), however, have shown that C^{14}-labeled 2-methyl-1,4-naphthoquinone administered to rats and chickens, is converted in the liver into a compound of the K_2 series, 2-methyl-3-geranylgeranyl-1,4-naphthoquinone, and the existence of the polyisoprenoid ubiquinones or coenzymes Q in the animal body is now firmly established.

A new alkali-labile, fat-soluble substance, showing a sharp absorption band at 275 mμ, was first detected in the livers of rats by Moore (*279*). Later (*279a*), it was found in pig and guinea pig livers, together with a second substance, separable on paper chromatograms, which also had an absorption peak at 275 mμ, and an additional inflection at 330 mμ. These two substances were designated SA and SC, respectively, by Morton and associates (*280*), and the former was recognized as a quinone (Q_{275}) by Crane *et al.* (*281*), who isolated crystalline material m.p. 48–49°, from beef heart mitochondria, and various electron-transporting particles derived therefrom. Detailed evidence for the structure (CLIII) of this remarkable quinone soon appeared from the Liverpool (*282*) and Wisconsin (*283*) schools, and was rapidly established by degradative studies in the laboratories of Hoffmann-La Roche (*284*) and Merck (*285*). As already mentioned, four other crystalline quinones of the same type were isolated from microorganisms within a short time, and these are now referred to as coenzymes Q, or ubiquinones, in the American and European literature, respectively. To distinguish individual members of the group, two systems of notation are in use (Table VIII) which indicate the number of carbon atoms (ubiquinones), or the number of isoprene units (coenzymes Q), in the side chain. Laboratory syntheses of ubiquinones (*30*), (*45*), and (*50*) have been achieved (*286, 286a, 286b*).

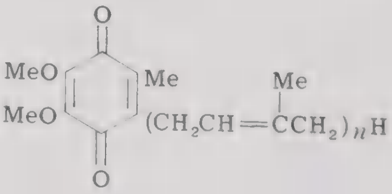
TABLE VIII
UBIQUINONE OR COENZYME Q NOTATION

Coenzyme Q	Source	M.P.[a]	n	Ubiquinone
Q_{10}	Beef heart	49.9°	10	(50)
Q_9	*Torula utilis*	45.2°	9	(45)
Q_8	*Azotobacter vinelandii*	37°	8	(40)
Q_7	*Torula utilis*	30.5°	7	(35)
Q_6	*Saccharomyces cerevisiae*	19–20°[b]	6	(30)

[a] Melting points are taken from reference (*283*) except where indicated.

[b] Melting point of ubiquinone (*30*) isolated from bakers' yeast and of synthetic material (*286*).

The coenzymes Q* are very similar in structure to quinones of the



(CLIII)

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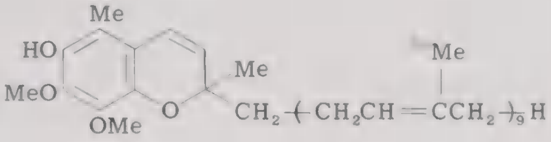
* Much additional information is given in a recent Ciba Foundation Symposium (*56*).

vitamin K_2 series elaborated by bacteria and to plastoquinone found in green leaves. Coenzyme Q_{10}, most commonly encountered in higher animals and plants, possesses a unique chain of ten isoprene units containing fifty carbon atoms. It is paralleled by the substance ubichromenol (formerly SC, see above) present in the liver and kidney of many species, human kidney being a rich source. Morton and co-workers (*287*) have shown recently that ubichromenol has structure (CLIV), in which part of the C_{50} side chain is incorporated in a heterocyclic ring. This compound is a cyclic isomer of ubiquinone$_{50}$, similar to solanachromene (LXIX), the cyclic isomer of plastoquinone, isolated from tobacco leaf.



(CLIV)

Ubiquinones have been found in all the common laboratory and domestic animals, but are not confined to mammalian tissues (see Table IX). In general, only coenzyme Q_{10} has been detected, but Folkers and his collaborators (*288*) found some indication, on paper chromatograms, of a second compound in the tissues of several species, this being the major component (possibly Q_9) in mouse carcass. Very recently Gloor and Wiss (*288a*) have found both Q_{10} and Q_9 in vitamin A-deficient rat liver, Q_9 predominating. In the wall-eyed pike coenzyme Q_{10} is replaced by Q_9 (*83*). For the isolation of crystalline material heart tissue is the most convenient: the procedure usually adopted is to hydrolyze the raw material with aqueous or ethanolic alkali, remove the unsaponifiable material by extraction with ether or hexane, which is then fractionated by column chromatography. Final purification is effected by crystallization from a polar solvent. By this general procedure the Hoffmann-LaRoche group obtained 37 gm. of pure crystalline ubiquinone$_{(50)}$ from 750 kg. of pig hearts (*284*). Other workers have isolated the same material from beef (*289*), horse (*290*), cod (*291*), and human (*288*) hearts.

In their initial communication on Q_{275}, Crane *et al.* (*281*) presented evidence for the participation of this new compound in the electron transport activities of mitochondria and mitochondrial fragments. This has been amply confirmed by much experimental work in the Wisconsin, and other, laboratories, and marks a considerable advance in our knowledge of electron transport. The concentration of coenzyme Q in

TABLE IX
Distribution of Coenzymes Q (Ubiquinones) in Animal Tissues[a]

Species	Organ	Coenzyme Q	Amount (mg./100 gm. wet tissue)	Reference
Cat	Liver	—	0.9, 1.3	(*299*)
Chick	Heart	Q_{10}	4.5	(*288*)
Cod	Heart	Q_{10}	4.86	(*90*)
	Liver	—	0.98	(*90*)
Cow	Heart	Q_{10}	7.3	(*83*)
	Liver	—	3.4	(*83*)
	Kidney	—	1.5	(*83*)
Dog	Liver	—	0.3, 2.0	(*299*)
Frog	Heart	Q_{10}	1.9	(*83*)
	Leg muscle	—	0.95	(*83*)
Guinea pig	Heart	—	1.3	(*83*)
	Liver	—	1.16	(*299*)
Horse	Heart	Q_{10}	9.5	(*290*)
	Liver	—	10.6, 1.2	(*299*)
Lamb	Heart	Q_{10}	7.2	(*83*)
	Kidney	—	4.7	(*288*)
	Leg muscle	—	5.2	(*83*)
	Spleen	—	1.1	(*300*)
Man	Heart	Q_{10}	6.3	(*288*)
	Liver	—	1.27	(*299*)
Mouse	Carcass	Q_9, Q_{10}	2.3	(*288*)
	Liver	—	4.1, 4.5	(*299*)
Ox	Heart	Q_{10}	6.6, 9.4	(*288*)
	Liver	—	1.15, 3.5	(*299*)
	Kidney	—	4.1	(*288*)
	Pancreas	—	1–2	(*300*)
	Spleen	—	0.9	(*300*)
	Striated muscle	—	0.12	(*288*)
Pig	Heart	Q_{10}	8.5	(*288*)
	Liver	—	2.4, 3.0	(*299*)
	Pancreas	—	1–2	(*300*)
	Spleen	—	1.9	(*300*)
Pullet	Kidney	—	4.6, 10.8	(*299*)
Rabbit	Heart	Q_{10}	9.5	(*288*)
	Liver	—	1.27	(*299*)
Rat	Heart	Q_{10}	14.0	(*288*)
	Liver	Q_9, Q_{10}	—	(*288a*)
Sheep	Liver	—	1.5, 3.3	(*299*)
	Pancreas	—	1–2	(*300*)
	Spleen	—	0.8	(*300*)
Turkey	Heart	Q_{10}	5.5	(*288*)
Wall-eyed pike	Skeletal muscle	Q_9	0.86	(*83*)

[a] Additional information is given in reference *56*.

different tissues is consistent with its respiratory function, e.g., the Q_{10} content of beef muscle (1.2 mg./kg. moist tissue) obtained from the neck muscle of steers, is approximately 1% of that found in beef heart tissue, which has a higher rate of respiration (*288*). High concentrations have been found in all mitochondrial sources examined, the quinone being localized almost entirely in a lipoprotein fraction (*292*). When mitochondria, or derived particles, are extracted with isooctane, the coenzyme Q is removed and simultaneously succinic oxidase activity is lost. This capacity can be restored by addition of coenzyme Q (*281*, *293*). (Other lipid components are also extracted by isooctane, and it may be necessary to add the Q-containing lipoprotein for complete restoration of activity.) Coenzyme Q (either particle-bound or externally added) is rapidly reduced by substrates (e.g., succinate or reduced diphosphopyridine nucleotide), and the reduction product is rapidly oxidized in the presence of mitochondrial enzymes, the oxidoreduction processes being affected in the usual way by conventional inhibitors of the electron transport system (*293*). Thus coenzyme Q is an active component of the electron transport particle, and accumulated evidence (*294*, *295*) indicates that it occupies a site in the succinic chain between cytochromes b and c. Coenzyme Q activity is limited to 2-methyl-5,6-dimethoxybenzoquinones, which have an isoprenoid side chain of at least ten carbon atoms at position 3 (*296*). Several other quinones, including vitamins K and tocopherolquinone, failed to restore succinic cytochrome c reductase and succinoxidase activity in mitochondria from which coenzyme Q_{10} had been removed by solvent extraction, although plastoquinone shows some degree of coenzyme Q activity (*76a*, *294*, *297*).

By studying the oxidoreduction reactions of coenzyme Q under steady-state conditions, Hatefi (*298*) has shown that it acts as an electron carrier in oxidative phosphorylation. Addition of inorganic phosphate stabilizes coenzyme Q in a reduced form (quinol phosphate), and this effect is reversed by adenosine diphosphate (ADP). In the terminal reaction ATP is formed by transfer of a high energy phosphate group to ADP, and the quinone carrier becomes available again for a repetition of the oxidative phosphorylation cycle.

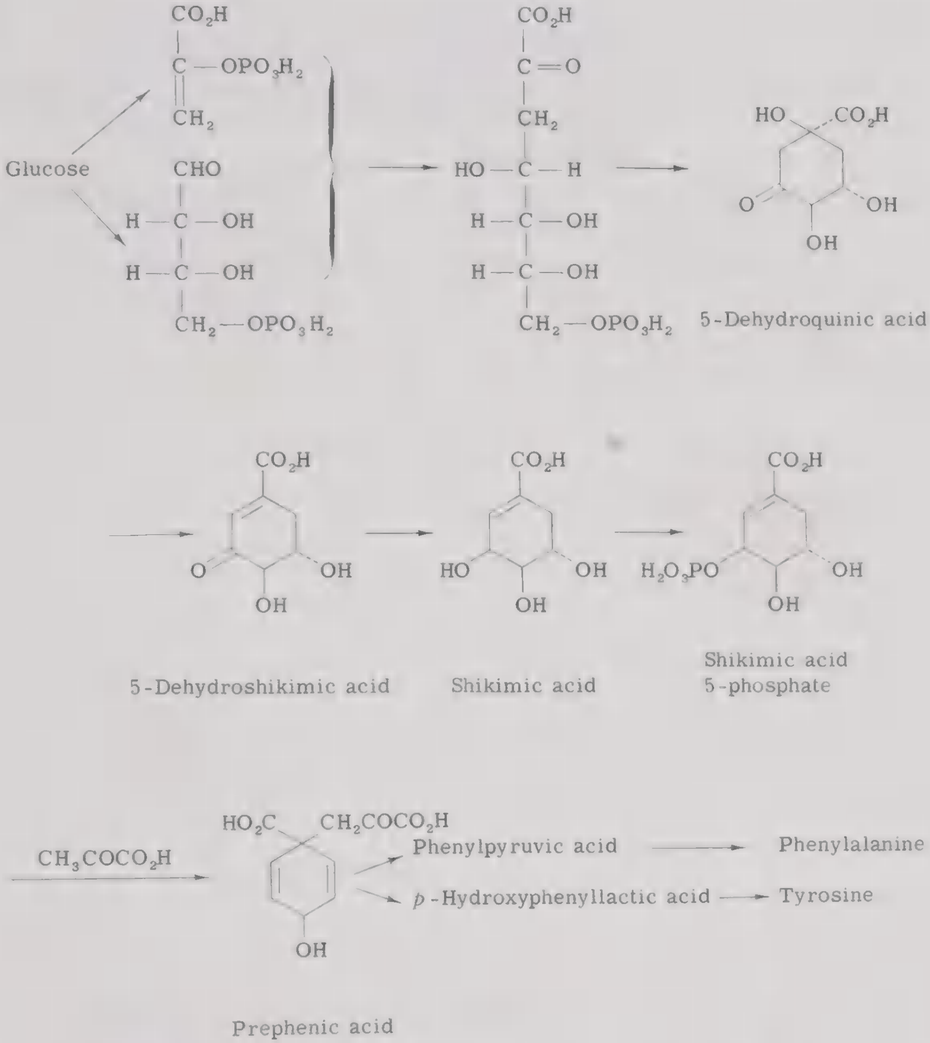
Martius and Nitz-Litzow (*301*) postulated that vitamin K is also concerned in oxidative phosphorylation, on the basis of experiments with chick and rat liver mitochondria. This view has not found general acceptance, and indeed there is no agreement on the existence of vitamin K in animal mitochondria. According to Dam and co-workers (*277*) it exists in beef liver mitochondria, but Lester and Crane (*83*) could find no trace in beef heart lipids, and other workers have failed to detect vitamin K in heart muscle preparations (*302, 303*), and in pig heart mitochondria (*304*). [The present position on the role of vitamin K in oxidative phosphorylation has been reviewed by Martius (*305*) and by Dam and Søndergaard (*305a*). For more detailed discussion of electron transport and oxidative phosphorylation see Vols. I and V of this work.] Whether or not vitamin K occurs in liver mitochondria, there is no doubt that vitamin K is present in liver where it appears to function as a coenzyme involved in the production of prothrombin and proconvertin which are essential for the coagulation of blood (*305a*). Its origin, however, is still uncertain. Presumably some is exogenous, but probably most of it is biosynthesized, in whole or in part, by intestinal bacteria. The experiments of Martius and Esser (*278*) show that a polyisoprenoid side chain can be added to a preformed 2-methylnaphthoquinone molecule in the liver, but the precise vitamin K compound(s) normally synthesized within the animal body are not known.

III. Biogenesis of Quinones

In recent years two major routes have been recognized for the biosynthesis of aromatic compounds from carbohydrates. They account for the formation of numerous natural products, including most of the quinones, but there are many other compounds that do not appear to fit into either of these schemes. For these, other modes of biogenesis must exist.

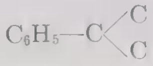
A. SHIKIMIC ACID ROUTE

The shikimic acid pathway leading to phenylalanine, tyrosine, and other aromatic acids is now firmly established by the studies of Davis, Ehrensvärd, and others. [As this is discussed elsewhere in this treatise (Vol. IV) and in several reviews (e.g., *153, 306, 307*), it will not be considered in detail here.] It was found that mutants of *Escherichia coli* (and other bacteria and fungi) required several aromatic amino acids for growth, all of which could be replaced by shikimic acid. Certain mutants accumulated shikimic acid, which could be isolated from the culture medium; others, which introduced an enzymatic block earlier in the reaction chain, accumulated precursors of shikimic acid. In this way, by working back along the biosynthetic route, using mutants to block each stage in turn, it was possible to establish the biogenetic sequence shown. Enzymatic analysis of the early stages of the aromatic biosynthesis showed that glucose is first transformed into C_3 and C_4 fragments which then combine to give a C_7 intermediate, followed by cyclization to 5-dehydroquinic acid. The specific enzymes concerned in the subsequent steps leading to shikimic acid have all been identified by Davis *et al.*, but the manner of its conversion to prephenic acid is



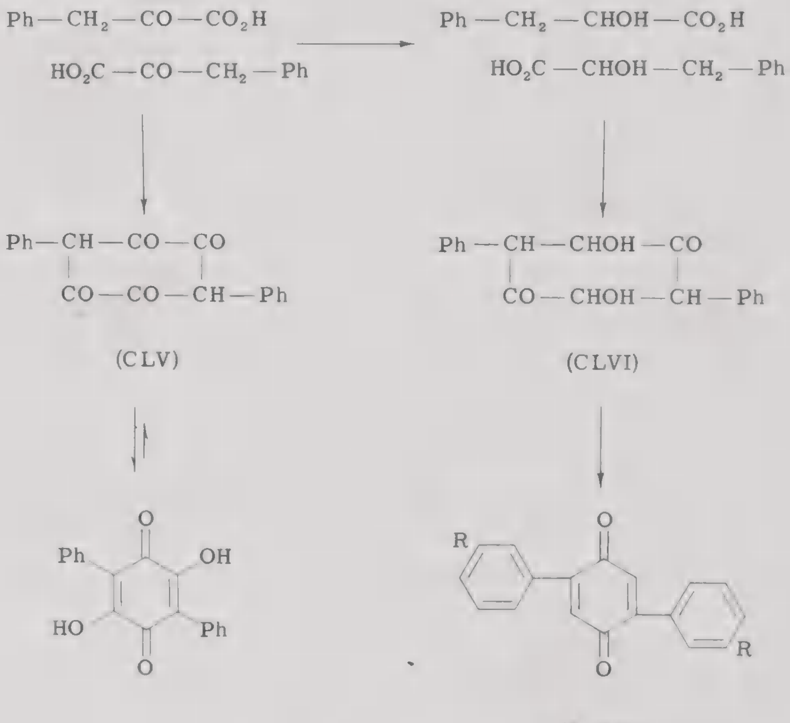
not yet elucidated. This latter intermediate is somewhat unstable and readily breaks down to phenylpyruvic acid below pH 6, but its conversion *in vivo* to aromatic acids is probably catalyzed by enzymes. As each step in the shikimic acid pathway can be reproduced *in vitro* with the aid of the appropriate enzyme, the biogenesis of aromatic compounds by this route, at least in microorganisms, is fully established.

The only evidence directly implicating shikimic acid in quinone biogenesis concerns volucrisporin (CLVII; R = OH) and, by implication, all the terphenylquinones. Mittal and Seshadri (*308*) suggested that these C_{18} compounds (and the closely related pulvinic acid pigments) might be derived from two C_6—C_3 forked units



$$C_6H_5—C\langle^{C}_{C}$$

such as 2-phenylglycerose. Read and Vining (*309*) thought that dimerization of two commonly available C_6–C_3 straight-chain intermediates was more probable, and have shown, in preliminary tracer studies, that highly active volucrisporin is produced when *Volucrispora aurantiaca* is grown in a nutrient medium containing C^{14}-labeled shikimic acid or L-phenylalanine. On the other hand neither labeled glycine, tyrosine, 3,4-dihydroxyphenylalanine, nor acetate, cinnamate and caffeate, were incorporated into the mold metabolite. This implied that the *m*-hydroxyl groups were introduced separately, and not derived from a dihydroxylated intermediate. Read and Vining therefore proposed the biogenetic scheme shown, in which two activated phenyllactic acid

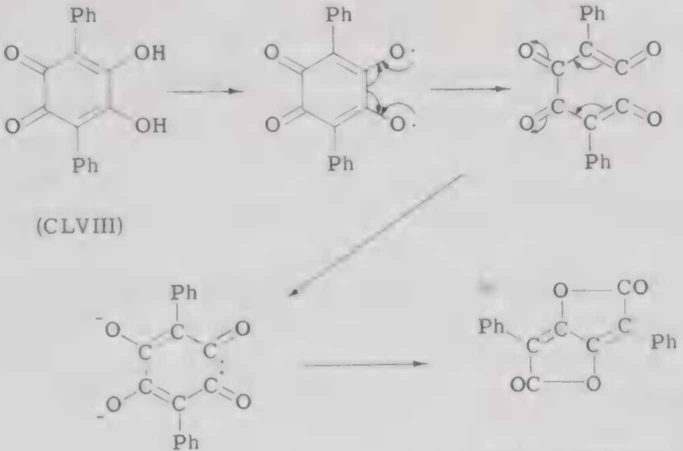


(CLV)

(CLVI)

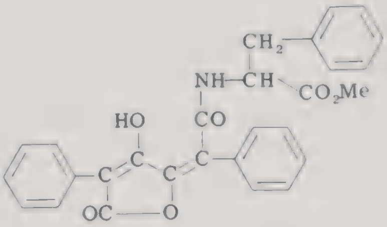
(CLVII)

molecules, formed by reduction of phenylpyruvic acid, condense to form the diketone (CLVI). Dehydration would then give *p*-terphenylquinone (CLVII; R = H), which could be converted into volucrisporin (CLVII; R = OH) by hydroxylation. A similar condensation of two activated phenylpyruvate units would give the tetraketone (CLV), tautomeric with polyporic acid. The other terphenylquinones (p. 657) produced by Basidiomycetes are hydroxylated derivatives of polyporic acid. It will be recalled that polyporic acid is also a lichen product and is found along with pulvinic acid pigments. The latter presumably arise by oxidation of (CLV) or (CLVI), very probably by initial hydrogen abstraction from the tautomeric form (CLVIII) of polyporic acid, as suggested by Mittal and Seshadri. It is of interest that one of the compounds of the pulvinic acid group, rhizocarpic acid (CLIX), produced by the lichen *Rhizocarpon geographicum* and other species (*310*) contains a phenylalanine residue.



(CLVIII)

Pulvinic dilactone



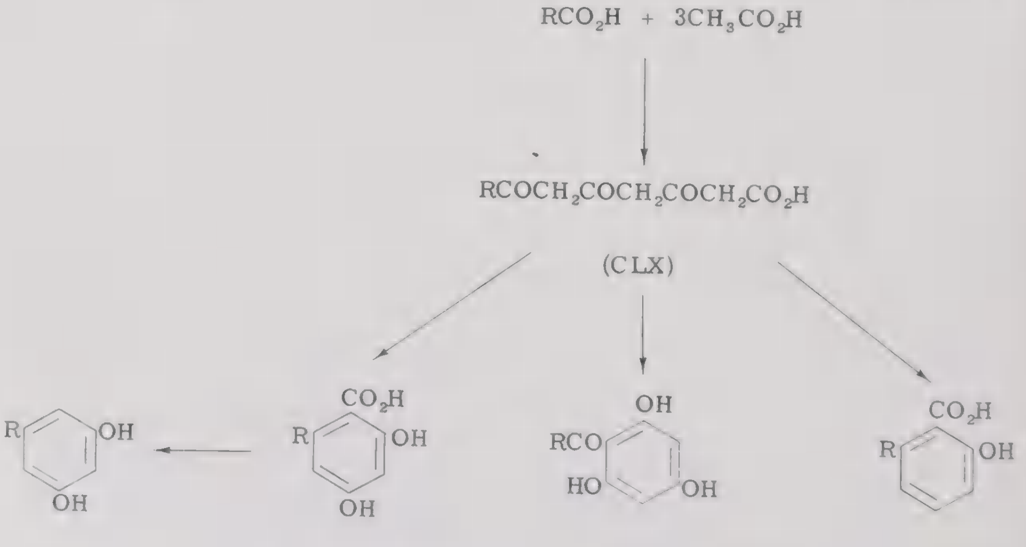
Rhizocarpic acid

(CLIX)

So far reference has been made only to microorganisms. However shikimic acid is probably of wide occurrence in higher plants (*311*), and evidence is accumulating that here also it is a source of aromatic compounds. Neish and co-workers (*312*) have shown in experiments with *Salvia splendens* and other plants, that labeled shikimic acid is the precursor of tyrosine and phenylalanine, which in turn is the precursor of cinnamic acid and various phenolic acids. Others have shown that shikimic acid is incorporated into the lignin of sugar cane (*313*), into caffeic acid in tobacco (*314*), and into quercetin in buckwheat (*314, 315*). As yet there is nothing which definitely suggests that any of the higher plant quinones are derived from shikimic acid, but our knowledge of the biogenesis of these compounds is woefully small.

B. Acetate Route

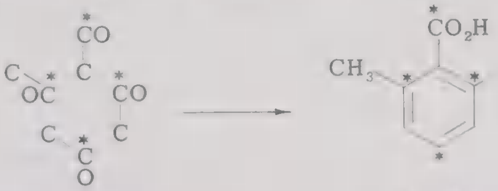
The idea that phenolic compounds might be evolved in nature by head-to-tail linkage of acetic acid units was first proposed by Collie (*316*) and was based on laboratory experiments in which he found that polyketones (e.g., diacetylacetone) could be condensed to form orcinol and other compounds related to natural products. At that time there was no means of testing this hypothesis, and it was forgotten. Similar views were expressed much later by Robinson (*317*), and more recently Birch (*318*) has extended and elaborated the acetate theory and has provided conclusive experimental evidence. The biosynthesis of fats and steroids by condensation of activated acetate units is now well established, and the most recent data on the formation of aromatic compounds by this route suggest a close parallel. In the formation of fatty acids (see Chapter 1, this volume) the carbonyl groups are eliminated by reduction and dehydration, but in phenol biogenesis most of the oxygen atoms remain attached to the carbon skeleton and can thus be recognized. Inspection of formulas suggested that many groups of phenolic substances, in particular, the fungal anthraquinones (*319*), were built up from acetate units, and moreover such scrutiny led to the discovery of erroneous structures and to the correct prediction of new ones. This gave strong support to the hypothesis prior to experimental verification. As Birch and Donovan (*320*) pointed out, the head-to-tail linkage of activated acetate units could lead to phenolic compounds in several ways. Extending the elaboration of a fatty acid RCO_2H (R contains an



(CLX)

(CLXI) (CLXII) (CLXIII) (CLXIV)

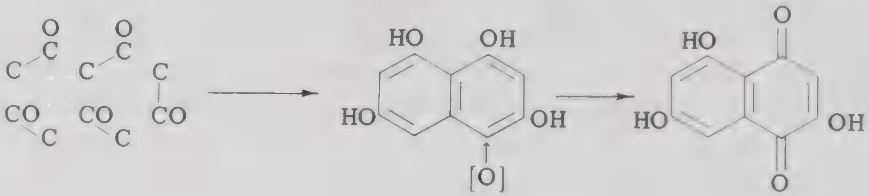
odd number of carbon atoms) by addition of unreduced acetate units could lead to a polyketone (CLX), from which orcinol (CLXII) or phloroglucinol (CLXIII) derivatives could arise by internal aldol or *C*-acylation reactions, respectively. Orsellinic acid (CLXII; R = Me) is a constituent of a large number of depsides and depsidones elaborated by lichens. Decarboxylation of (CLXII) could give the orcinol (CLXI); and reduction, followed by dehydration, of one of the carbonyl groups in (CLX) not involved in the cyclization, would lead to (CLXIV). It will be noted that all the aromatic compounds are formed by straightforward reactions for which *in vitro* and *in vivo* analogies exist. 6-Methylsalicylic acid (CLXIV; R = Me) was the first compound used by Birch *et al.* (*321*) to test the acetate hypothesis. Microorganisms are very convenient for studies of this kind as they can be readily grown in the laboratory under controlled conditions. Experiment has shown that many organisms initially break down glucose to acetate (presumably via the Embden-Meyerhof pathway), so that the biosynthesis of aromatic compounds from the latter can be followed by feeding experiments in which carbohydrate is replaced by labeled sodium acetate. *Penicillium griseofulvum* produces 6-methylsalicylic acid when grown on a glucose medium, and when fed with sodium acetate (labeled with C^{14} in the carboxyl group) the aromatic compound produced was radioactive. Isotopic distribution analysis showed that the



(CLXV)

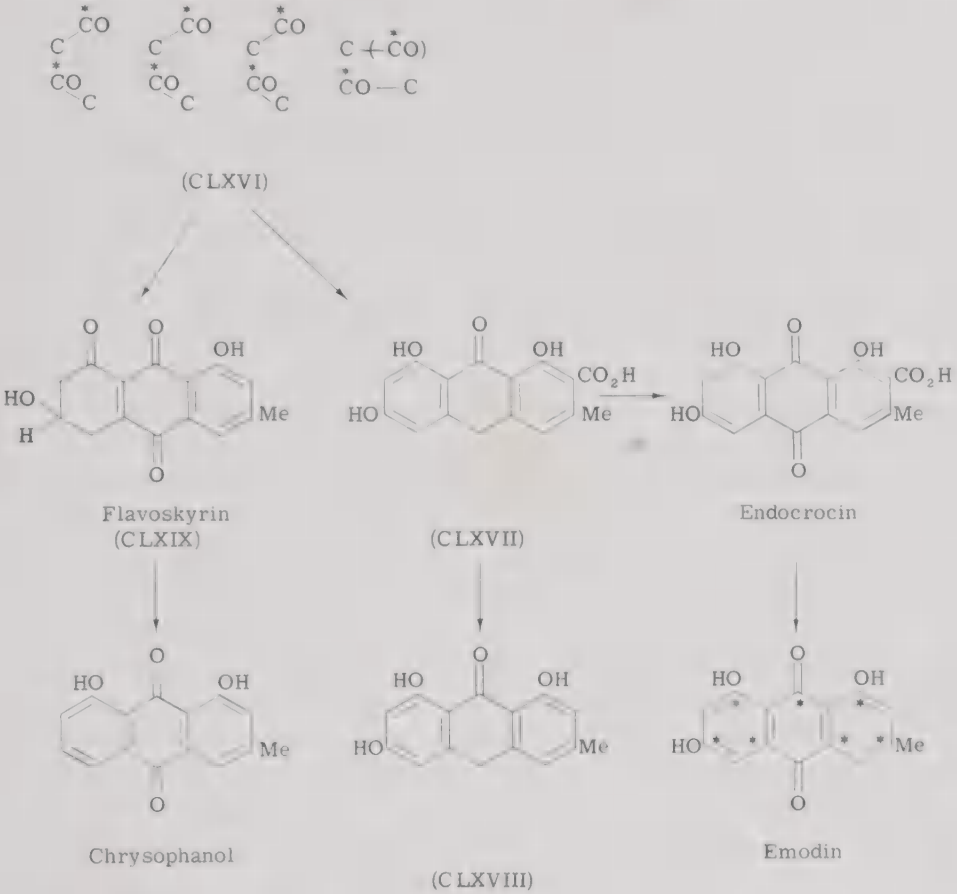
6-methylsalicylic acid (CLXV) was labeled with C^{14} as predicted on the basis of head-to-tail linkage of acetate units. This has been corroborated by Tanenbaum and Bassett (*322*). Very recently, Gatenbeck and Mosbach (*323*) have obtained radioactive orsellinic acid by growing *Chaetomium cochliodes* in a medium containing $CH_3C^{14}OO^{18}Na$. The incorporation of O^{18} fully confirmed its biogenesis by direct condensation of activated acetate units. Moreover the O^{18} content of the carboxyl group of the orsellinic acid was about half that of the hydroxyl groups, in agreement with successive condensation of acetate units via acetyl-coenzyme A, the final step being hydrolysis of the terminal thiolester.

The simple acetate theory has been extended in several ways in order to embrace a much wider range of natural products. In addition to the cyclization of β-polyketones formed by head-to-tail linkage of acetate units, additional reactions may take place which bring about (*a*) elimination of oxygen (as in the formation of 6-methylsalicylic acid); (*b*) introduction of oxygen; (*c*) introduction of substituents, notably methyl, isopentenyl, and related groups; and (*d*) rearrangement of the carbon skeleton. Introduction of oxygen to aromatic systems is a common biological process (*153*) and of particular importance in the biogenesis of quinones. Flaviolin is a typical example; it was shown by Astill and Roberts (*324*) to be 2(or 3),5,7-trihydroxy-1,4-naphthoquinone, having a resorcinol structure in one ring, characteristic of acetate-derived compounds. This quinone could be formed from five acetate units, assuming the additional introduction of oxygen, as indicated below. On this basis Birch and Donovan (*325*) predicted that flaviolin would be the 2,5,7-isomer, and this was confirmed by synthesis (*319, 326*). Similarly, the position of the methoxyl group in the fungal naphthoquinones javanicin and fusarubin (XCVI, XCVII) has not been established, but its location at C-7 is predicted on the basis of acetate biogenesis (*325*).



Flaviolin

A glance at the structures of the numerous anthraquinones produced by fungi (p. 661–663) at once suggests that these compounds are biosynthesized from acetic acid. Condensation of eight acetate units according to the simple theory would lead to the formation of the anthrone (CLXVII). This product is not known as a fungal metabolite, but the two quinones endocrocin and emodin, formed by subsequent oxidation and decarboxylation, are both formed by an ultraviolet mutant of *Penicillium islandicum* (*327*). Endocrocin also occurs with physcion (the 6-methyl ether of emodin) in the lichen *Nephromopsis endocrocea.* Emodin is produced only in traces by the wild type of *P. islandicum,* but skyrin (5,5′-diemodin, p. 664) is formed in abundance, and this was selected by Gatenbeck (*328*) for biogenetic study. In feeding experiments using carboxyl-labeled acetate as carbon source, radioactive skyrin was isolated from the mycelium and then reduced with sodium dithionite to emodin, before subjection to isotopic distribution analysis. The results were fully in accord with the distribution of C^{14} predicted by theory. The other fungal anthraquinones are variants of the emodin


(CLXVI)

Flavoskyrin (CLXIX)

(CLXVII)

Endocrocin

Chrysophanol

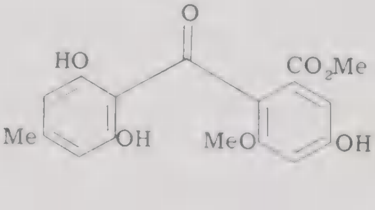
(CLXVIII)

Emodin

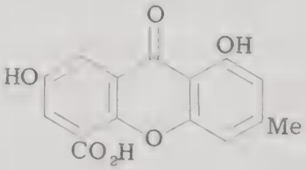
structure, characterized by the absence of oxygen in "expected" positions, or possessing "additional" oxygen substituents, or both. These can be accounted for by appropriate oxidations, reductions, and dehydrations, as already mentioned, and in two cases, helminthosporin (*329*) and islandicin (*330*), biosynthesis from acetate units has again been established by isotopic experiments. Gatenbeck (*330*) has gone further by using $CH_3C^{14}OO^{18}H$ in feeding experiments with *P. islandicum*. In this way it was possible to distinguish between the oxygen atoms of islandicin that are derived from acetate carboxyl oxygen and those that are introduced by secondary oxidation. These findings were also in agreement with expectations based on the acetate theory.

A particularly interesting metabolite of *P. islandicum* is flavoskyrin (CLXIX). This is readily dehydrated to chrysophanol *in vitro* (*331*) and appears to represent an intermediate step in the process of aromatization. However, dehydration *in vivo* may not take place, for recent investigations by Gatenbeck (*332*) on the pigments of *P. islandicum* show that rubroskyrin and luteoskyrin (CXVI and CXVIII), hydrated forms of iridoskyrin analogous to flavoskyrin, are *not* precursors of iridoskyrin. Nor, conversely, is iridoskyrin a precursor of its hydrated forms, whence it follows that the immediate precursors of the diquinone, and of its hydrated forms, are different. These findings emphasize our general ignorance of the precise pathways by which two-carbon units are built up to form complex ring systems. The biosynthesis from labeled precursors of certain quinones, and a variety of other mold metabolites, giving products having the predicted isotopic distribution pattern, is conclusive proof of the acetate hypothesis, but further study is now required to put it on a sound biochemical basis by isolation of intermediate products, if possible (see below), and identification of the enzymes that promote the sequence of reactions.

The biogenesis of the fungal anthraquinones has provoked considerable speculation. Seshadri has discussed the formation of fungal (*333*) and lichen (*334*) products, and considers a C_8 unit (orsellinic acid) to be of great importance in the formation of quinones (*335*) as well as the depsides and depsidones. This, of course, fits into the acetate theory. Two C_8 units are required to construct the anthraquinones, but although it is possible to formulate reaction schemes there is no evidence whatsoever that orsellinic acid, or related compounds, are actual intermediates. Raistrick (*336*) pointed out some years ago that a number of simple phenolic acids produced by molds are possible intermediates in the biogenesis of anthraquinones, but it has been shown (*336a*) that 3-hydroxyphthalic acid [yet another acetate-derived metabolite of *P. islandicum* (*337*)], is not a precursor of islandicin, and 6-methylsalicyclic acid, conceivably a precursor, completely inhibits anthraquinone formation by this organism. It has also been suggested that mold metabolites such as sulochrin and pinselinic acid arise by condensation of simple phenolic acids, such compounds being regarded as the products of incomplete anthraquinone biosynthesis. A tetrahydroxymethylbenzoylbenzoic acid (*337a*) recently obtained from *P. islandicum* has every appearance of being an anthraquinone intermedi-



Sulochrin

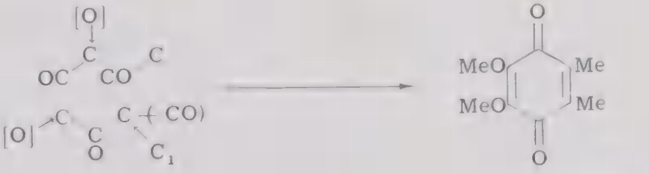


Pinselinic acid

ate. However, when this compound was labeled with C^{14} and fed to *P. islandicum*, none of the anthraquinones produced were radioactive (*336a*). The complete failure to detect precursors, together with Gatenbeck's demonstration, already referred to, that the closely related anthraquinones formed by *P. islandicum* do not have the same immediate precursors, has led Ehrensvärd and Gatenbeck (*336a*, *338*) to the view that intermediates do not exist above the enzyme level. They suggest that anthraquinone formation proceeds in three stages: in the first stage acetate units are arranged in a regular manner within an enzyme complex, this is followed by random condensation of carbonyl groups with methyl and methylene groups leading to the formation of different products simultaneously, which, in the third stage, separate from the enzyme surface as definite chemical entities. In this "all-or-nothing" process there are no intermediates, in the ordinary sense, between a C_2 unit and a complete $15 \times C_2$ compound (in the case of a dianthraquinone), and sulochrin, pinselinic acid, etc., are regarded as secondary oxidation products derived from anthraquinones.

Probably all the fungal benzoquinones are acetate derived, and compounds of the orsellinic acid type are postulated intermediates. Their conversion to quinones would require nuclear oxidation, methylation, decarboxylation, and similar reactions, which Seshadri has shown to be feasible *in vitro*, by laboratory syntheses of several benzoquinones along these lines (*339*). That such reactions ensue *in vivo* has been confirmed by biological evidence concerning the biogenesis of aurantiogliocladin (LXXX). This quinone has two *C*-methyl and two *O*-methyl groups, and an additional *C*-methyl group has to be introduced during its biosynthesis [extension (*c*) of the simple acetate theory (p. 702)]. In separate experiments, Birch and co-workers (*340*) grew the mold on media containing labeled acetate and labeled formate. The radioactive products were then combined, reduced to the quinol, and the isotopic distribution pattern was determined by appropriate degradations.

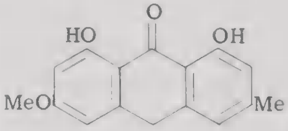
The results support the scheme of biogenesis from acetate units shown, which requires nuclear oxidation at two carbon atoms, decarboxylation, and *O*- and *C*-methylation. *C*-Methyl groups may be



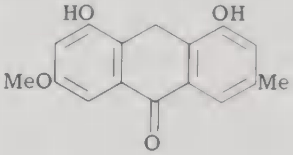
Aurantiogliocladin

introduced before or after ring formation, the process being strictly analogous to *O*-, *S*-, and *N*-methylation, in which the methyl group is transferred as CH_3^+ from an onium compound derived from methionine or choline, to the methyl acceptor. (For a full discussion readers are referred to Chapter 5 in Vol. I of this work.)

The fourth extension of the acetate theory—rearrangement of the carbon skeleton—has not been definitely established in quinone biogenesis. A possible example referred to earlier is the formation of *both* anthrones of physcion by fungi and higher plants. On the simple theory the anthrone (CLXX) would be expected, but not the isomer (CLXXI).

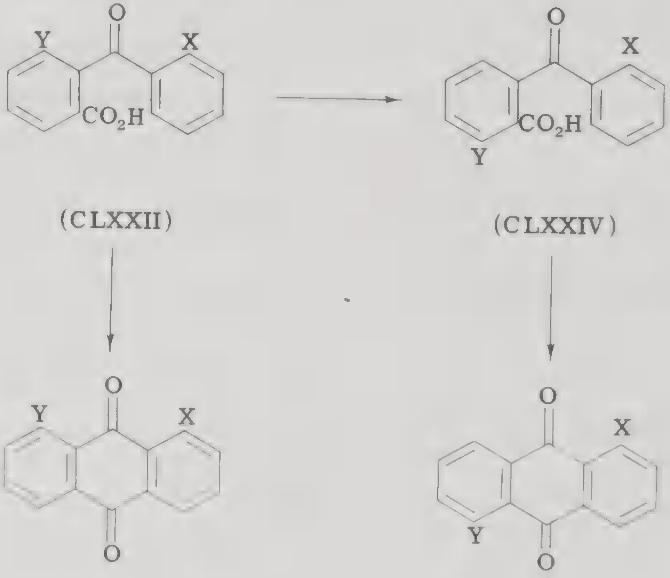


(CLXX)



(CLXXI)

Cyclization of an *o*-benzoylbenzoic acid normally leads directly to an anthraquinone (e.g., CLXXII→CLXXIII). However cases are known where this is preceded by a rearrangement, (CLXXII→CLXXIV→CLXXV), the first step being attack by a carbonium ion (*341*) or

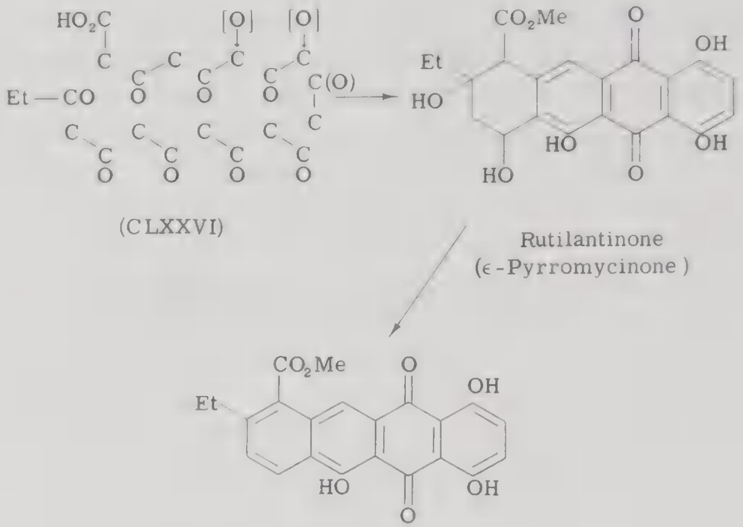


carboxyl radical (*342*) derived from the carboxyl group, on the carbon atom in the other ring to which the keto group is attached. A rearrangement of this type might account for the formation of the two physcion anthrones and for other anthraquinones which appear to be acetate derived but have a "wrong" substitution pattern in one ring.

The isotopic studies referred to above, and many others, seem to indicate that the acetate route is the principal one used by fungi in the biosynthesis of aromatic compounds. Most fungal quinones fit into this scheme [6-methyl-1,4-naphthoquinone (XCIII) and boletol (CXII) may be exceptions], but other modes of biogenesis are, of course, possible. Tanenbaum and Bassett (*343*) have found that *Penicillium patulum* produces a variety of acetate-derived aromatic compounds (e.g., 6-methylsalicyclic acid) and also pyrogallol, which appears to arise by the shikimate pathway. The biosynthesis of quinones by the latter route is obviously possible, as we have seen already. It will be recalled that tetrahydroxybenzoquinone is formed by bacterial oxidation of *myo*-inositol (p. 675), which suggests other possibilities.

Little experimental evidence is available on the biogenesis of quinones other than those formed by fungi, but there is structural evidence which suggests that many other quinones are built up from acetate units. The fact that nearly all natural derivatives of naphthalene, and more particularly anthracene, are quinones is perhaps a pointer in this direction, since the acetate pathway leads directly to polyphenolic derivatives (especially 9-anthrones) which are readily convertible to quinones without the aid of enzymes. It can be assumed that lichen anthraquinones (pp. 665–666) are formed from acetate precursors; this route appears to operate in bacteria. The pyrromycinones and related pigments produced by Streptomycetes, are striking examples of aromatic compounds formed by head-to-tail linkage of acetate units (CLXXVI) (*166*), and the structure determinations of Brockmann (*164*), Prelog (*165*), and Ollis (*166*) were based, in part, on the acetate theory. ϵ-Pyrromycinone containing a hydroaromatic ring with all the oxygen atoms still in position, is presumably an intermediate stage en route to the fully aromatic η-pyrromycinone. The tracer studies of Ollis and co-workers (*344*) have established that the carbon skeleton of ϵ-pyrromycinone is of acetate origin but the *C*-ethyl group (see CLXXVI) is actually derived from propionate. This is the first clear demonstration of mixed acetate-propionate biogenesis and it exemplifies a further modification of the simple acetate theory.

In higher plants, the occurrence of anthraquinones of the emodin type in the Rhamnaceae, Polygonaceae, and other families, implies that these compounds originate in the same way as in the fungi, and the frequent occurrence of the corresponding anthrones supports this view. Birch (*345*) has pointed out that both anthraquinones and phenanthrene-

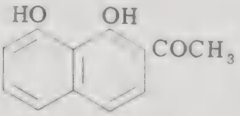


(CLXXVI)

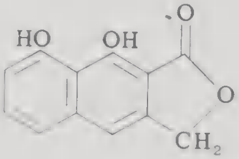
Rutilantinone
(ϵ-Pyrromycinone)

(η-Pyrromycinone)

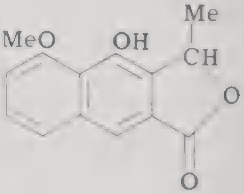
quinones, which occur together in *Rumex chinensis*, can be derived from the same polyketo chain (CLXVI) by appropriate condensations. Of particular interest is the complex quinone hypericin (XXXVI) which is probably derived from emodinanthrone (CLXVIII) by stepwise oxidative coupling of each ring. 10,10′-Diemodin-9-anthrone has not been detected (cf. penicilliopsin, p. 662), but the corresponding dehydrodianthrone and helianthrone were found with emodinanthrone in *Hypericum hirsutum* (*17*). [It has been reported that hypericin and emodin occur in the fungus *Polystictus versicolor* (*108*).] The occurrence (*346*) of the ketone (CLXXVII) in the bark of *Rhamnus frangula* (a source of emodin) is of interest, as its 3,6-dimethyl derivative was synthesized by Collie (*316*) from diacetylacetone in his original work



(CLXXVII)



(CLXXVIII)



(CLXXIX)

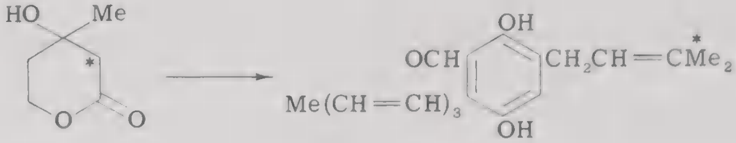
which led to the acetate hypothesis. Other nonquinonoid naphthalene compounds (*68*) that obviously originate from acetate units are the sorigenins (CLXXVIII; R = OMe and H) found in the bark of *R. japonica*, and eleutherol (CLXXIX), which accompanies the eleutherins

(LI) in *Eleutherine bulbosa.* Naphthoquinones of the plumbagin type (XLVIII), the benzoquinones with long alkyl side chains, like embelin (LXIII) (the side chains all contain an *odd* number of carbon atoms), can also be fitted into the acetate scheme without difficulty.

In the animal kingdom evidence concerning the biosynthesis of quinones is badly needed. Structural comparisons, and the existence of polyhydroxylated compounds, suggest that an acetate origin is plausible in many cases, but it must be emphasized once again, that other biogenetic schemes must exist. The polyhydroxynapthoquinones in echinoids are conceivably derived from acetate units, assuming that oxygen can be introduced at several points. A common origin for flaviolin (XCIV) and spinochrome N (CL) seems likely. It has already been noted that the anthraquinones evolved by crinoids (p. 690) are similar in structure to some of the lichen quinones. In the insect world, the anthraquinones can be fitted into an acetate scheme (the formation of erythrolaccin would require rearrangement of one ring) and so can the aphin pigments (*345*) (p. 685). The latter view is supported by the fact that the xanthoaphins and chrysoaphins, the hydrated forms, and precursors, of the fully aromatic erythroaphins, are similar to the type of structure elaborated by bacteria and fungi. Moreover, pigments of the erythroaphin type are actually produced by fungi (*234*).

C. ISOPRENOID QUINONES

It has already been noted that thymoquinone (p. 648), perezone (LXI), and the tanshinones (p. 643) are products of terpene metabolism. A number of other quinones are partly terpenoid in character, and belong to a fairly extensive group of natural products in which an isoprene or polyisoprene unit is attached to an aromatic or heterocyclic ring system. The most obvious examples are the ubiquinones, vitamins K and plastoquinone; others are lapachol, lomatiol, and dunnione (XLI–XLIII), and the quinols auroglaucin, flavoglaucin, and pyrolatin (LXXXII, LVII). Birch (*318*) suggested that these hydrocarbon side chains were introduced biosynthetically by a *C*-isopentenylation reaction subsequent to ring formation, the process being analogous to *C*-methylation and closely related to that involved in the junction of isopentene units in the formation of terpenes. Evidence supporting this view has been obtained and it is very striking that the quinones most widely dispersed in plants and animals, are of this type. When Birch *et al.* (*347*) cultured *Aspergillus novus* in a medium containing 2-C^{14}-mevalonic lactone, the auroglaucin which they isolated was radioactive, the activity being confined almost entirely to a terminal carbon atom as shown be-



Mevalonic lactone Auroglaucin

low. (It was also shown by growing *A. novus* in a medium containing labeled acetate that the main skeleton of the molecule is built up from acetate units. The formation of auroglaucin requires considerable modification of the simple acetate hypothesis.)

In the animal organism, Gloor and Wiss (*348*) have shown that labeled mevalonic acid is incorporated into ubiquinone$_{(50)}$ in the liver of the rat, it being assumed that the radioactivity was confined to the isoprenoid side chain on the grounds that biosynthesis of a benzene or benzoquinone ring in higher animals has never been demonstrated. There is, however, the possibility that the ubiquinone may have been formed by intestinal microorganisms. In subsequent experiments with vitamin A-deficient rats it was found that mevalonic acid was incorporated into both ubiquinone$_{(50)}$ and ubiquinone$_{(45)}$, the latter predominating (*288a*). Gloor and Wiss (*348*) also established, by administering labeled α-tocopherol and labeled 2-methyl-5,6-dimethoxybenzoquinone, that these were not precursors of the quinone ring, as the ubiquinone subsequently isolated was not radioactive. The second case was somewhat unexpected as Martius and Esser (*278*) had previously shown by treating rats and chickens with 2-methyl-C^{14}-1,4-naphthoquinone, that it is converted by these animals into a quinone of the vitamin K_2 series (2-methyl-3-geranylgeranyl-1,4-naphthoquinone). This same quinone is also produced *in vitro* by liver homogenates (chicken) from 2-methylnaphthoquinone and mevalonic acid. More recently, Martius (*349*) has found that vitamin $K_{2(20)}$ is biosynthesized in man from 2-methyl-1,4-naphthoquinone. The biogenesis of terpenes is discussed elsewhere in this volume[*] and it will suffice here to refer to the recent work of Lynen (*350*), who has established that isopentenyl pyrophosphate (itself derived from mevalonic acid 5-pyrophosphate) functions as an "active isoprene" in the biosynthesis of terpenoid compounds. Isopentenyl pyrophosphate isomerase, derived from yeast, converts isopentenyl pyrophosphate (CLXXX) into dimethylallyl pyrophosphate (CLXXXI) from which polyisoprene structures are built up with the aid of synthetase enzymes.

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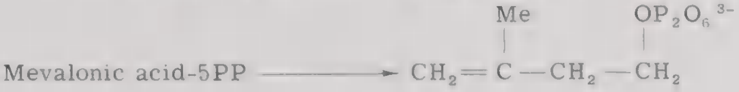
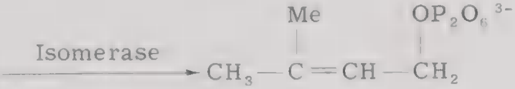
[*] See Chapter 11 in this volume.



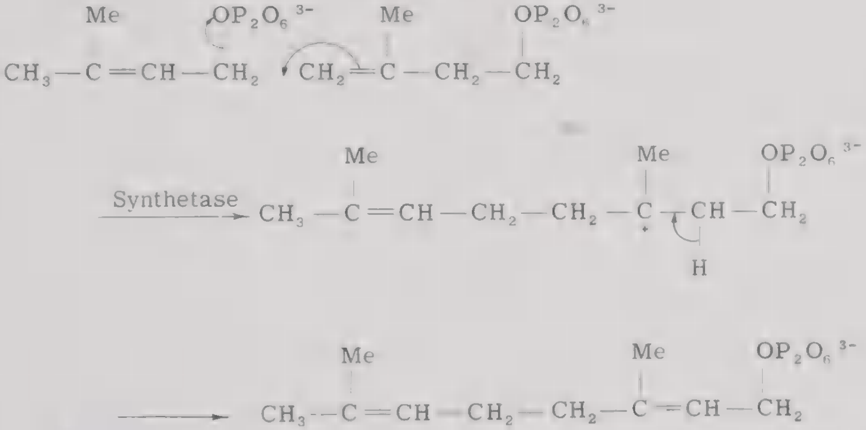
$$\text{Mevalonic acid-5PP} \longrightarrow CH_2{=}\overset{\overset{\large Me}{|}}{C}{-}CH_2{-}\overset{\overset{\large OP_2O_6^{\,3-}}{|}}{CH_2}$$

(CLXXX)



$$\xrightarrow{\text{Isomerase}} CH_3{-}\overset{\overset{\large Me}{|}}{C}{=}CH{-}\overset{\overset{\large OP_2O_6^{\,3-}}{|}}{CH_2}$$

(CLXXXI)

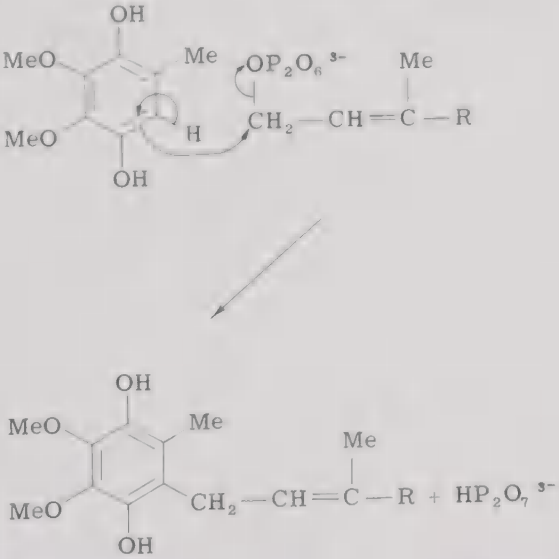


$$CH_3{-}\overset{\overset{\large Me}{|}}{C}{=}CH{-}\overset{\overset{\large OP_2O_6^{\,3-}}{|}}{CH_2} \quad CH_2{=}\overset{\overset{\large Me}{|}}{C}{-}CH_2{-}\overset{\overset{\large OP_2O_6^{\,3-}}{|}}{CH_2}$$

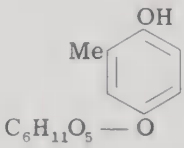
$$\xrightarrow{\text{Synthetase}} CH_3{-}\overset{\overset{\large Me}{|}}{C}{=}CH{-}CH_2{-}CH_2{-}\overset{\overset{\large Me}{|}}{\underset{+}{C}}{-}\underset{\underset{\large H}{|}}{CH}{-}\overset{\overset{\large OP_2O_6^{\,3-}}{|}}{CH_2}$$

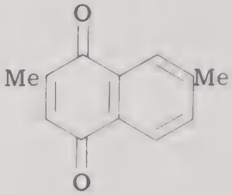
$$\longrightarrow CH_3{-}\overset{\overset{\large Me}{|}}{C}{=}CH{-}CH_2{-}CH_2{-}\overset{\overset{\large Me}{|}}{C}{=}CH{-}\overset{\overset{\large OP_2O_6^{\,3-}}{|}}{CH_2}$$

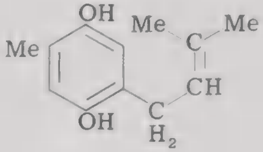
Isopentenylation of a quinol, e.g., in ubiquinone biosynthesis, is formulated as shown.

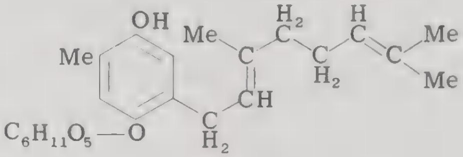


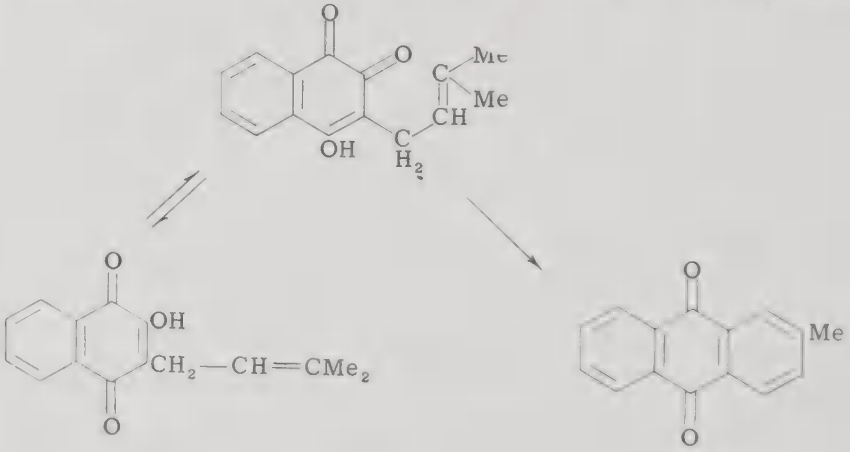
It may be, as Robinson (*317*) suggested, that certain naphthalene derivatives are biosynthesized by union of a benzenoid compound with a C_5 unit. This is possible in *Pyrola* spp. Homoarbutin (C_7) and chimaphilin (C_{12}) both occur in *P. incarnata,* and pyrolatin is found in *P. japonica.* The quinol (CLXXXII), similar to flavoglaucin, is not known but is a possible precursor of chimaphilin, and a similar cyclization of the C_{10} side chain of pyrolatin onto the benzene ring could lead to a naphthoquinone of the alkannin type (LII). Of particular interest is the co-occurrence of lapachol and 2-methylanthraquinone (tectoquinone) in *Tectona grandis* (Verbenaceae), and it is not difficult to visualize the formation of the anthraquinone from the naphthoquinone (25). Tectoquinone is accompanied in teakwood by 3-hydroxy-2-methylanthraquinone (also found in the Rubiaceae), and it could be that the numerous rubiaceous anthraquinones not readily accommodated by the acetate theory are biosynthesized in this manner.


Homoarbutin


Chimaphilin


(CLXXXII)


Pyrolatin


Lapachol

Tectoquinone

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CHAPTER 13

Melanins

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I. Introduction

The chemical investigation of natural pigments has a long and, on the whole, successful history. As can be seen from other chapters in this treatise, coloring matters of relatively low molecular weight which are easily isolated and purified have received most attention, and much less is known about the highly intractable, polymeric, melanins. Nevertheless, from the standpoint of comparative biochemistry the melanins are of the greatest interest, being responsible probably for most of the black, gray, and brown pigmentation so widely distributed in animals and plants. The enzyme system responsible for melanogenesis evidently appeared at an early stage of evolution although its purpose seems to have changed with later developments. Chordate melanins have a mainly protective function, e.g., in shielding underlying tissue from shortwave irradiation, in heat control, and in adaptive coloration, but the significance of melanins in plants and the more primitive animals is still obscure. The existence of melanins in human skin and hair, and its association with certain malignant tumors, is of obvious interest and importance, and mammalian melanism in consequence has been subjected to particularly intensive study, more especially by geneticists and cytologists (*1*).

Despite an enormous volume of biological research, melanins remain somewhat ill defined and are sometimes loosely described as pigments of high molecular weight formed by the enzymatic oxidation of phenols.

This definition is much too wide however, as it includes a whole range of products derived from a great variety of substrates that may have little structural relationship. The term melanin appears to have been used first with some precision by Fürth and Schneider (*2*) in respect of the black precipitate they obtained by the action *in vitro* of insect tyrosinase on tyrosine. The fact that the general properties and the carbon, hydrogen, and nitrogen analyses of this material were in approximate agreement with those reported for natural pigments obtained from animal hair, melanomas, *Sepia* black, etc., implied that these natural pigments also were products of the tyrosine-tyrosinase reaction. This is now generally accepted, and the term melanin is here restricted to the pigments formed by tyrosinase oxidation of tyrosine and such closely related compounds as β-(3,4-dihydroxyphenyl)alanine (dopa), tyramine, and 3-hydroxytyramine [β-(3,4-dihydroxyphenyl)ethylamine]. This is still not a satisfactory definition, but it must suffice until more is known of the structure of tyrosine melanin. Thus melanins, so defined, are nitrogenous polymers, the monomer being very probably an indole unit, but as the structure may well be irregular and the natural melanins are frequently conjugated with protein, a precise chemical definition may not be possible. Synthetic melanins can be obtained either by *in vitro* oxidation of tyrosine with tyrosinase or by autoxidation, but the latter process is not the same as natural melanogenesis. The structural relationship between the natural and synthetic products is discussed in Section IV.

Not only is the structure of natural melanin unknown, but there is still uncertainty about its elementary composition. The main difficulty, apart from the generally intractable nature of the material, lies in separating the pigment from concomitant protein. Since melanin is soluble in aqueous sodium hydroxide it can be isolated from tissue by alkaline extraction and recovered by acidification of the dark solution. Extraneous matter may be removed by solvent treatment, and separation from protein may be effected by prolonged hydrolysis with strong acids. Obviously the details vary according to the source of the pigment. The numerous analyses that have been reported vary over a wide range (some examples are noted on p. 747); it will suffice to mention two recent results concerning *Sepia* melanin, which occurs as a suspension of black granules in the ink sac of the cuttlefish. Mason *et al.* (*3*) reported the absence of admixed proteins in the ink, but when the melanin was hydrolyzed with 6 *N* HCl at 125° for 36 hours, at least twelve amino acids were liberated. Nicolaus and co-workers (*4*) obtained highly purified *Sepia* melanin by washing with dilute hydrochloric acid, followed by exhaustive extraction with hot acetone and then prolonged hydrolysis (150 hours) with boiling, concentrated hydrochloric acid. The melanin was finally obtained as an ashless black powder containing C, 64.08; H, 3.00; N, 8.52; and S, 0.2%. The trace of sulfur remaining after such vigorous treatment suggests that the protein is bound to the pigment by an S-linkage.

Mammalian melanin is deposited in specialized cells (melanocytes) in the form of granules. These are complex structures, largely protein in nature, associated with various oxidase systems, including tyrosinase (*5*). The enzymes present partly account for the relatively high concentration of heavy metals (iron, copper, zinc) normally encountered, although there is also evidence that natural melanins can combine with heavy metals (with consequent changes in shade and intensity) (*6*). From electron micrographs of granules from various sources (human skin, beef choroid, Harding-Passey melanoma, etc.) Mason and coworkers (*7*) deduced that they existed as formed elements with a limited number of rod ($0.1 \times 0.4\mu$ to $0.18 \times 0.6\mu$) or spherical (0.2–2.0μ diameter) shapes. A more recent study by Laxer *et al.* (*8*) of the granules in pigmented hair (sheep, dog, horse, human, etc.) has shown that they have a composite structure made up of a colorless matrix upon which a sheath or envelope of melanin is deposited. On bleaching with alkaline hydrogen peroxide the melanin is removed, leaving a colorless granule, similar to the original, but smaller in size. On treatment with reducing agents (dithionite, ascorbic acid) the pigment fades to a light tan which reverts to black on reoxidation. Undoubtedly melanins exist in different states of oxidation; this accounts, in part, for the natural variations in shade although the actual number and disposition of the granules is probably more important.

No satisfactory tests are available for the identification of melanins. Alkali solubility and reversible reduction merely indicate phenolic and quinonoid properties, respectively, and none of the histochemical tests are specific (*9*). In general, if a dark pigment is insoluble in ordinary solvents,* is decolorized by oxidizing agents (chromic acid, hydrogen peroxide, etc.), and reduces ammoniacal silver nitrate, it is deemed to be a melanin. These inadequate diagnostic properties are strengthened if the pigment occurs in the form of granules and is associated with tyrosinase. However, the tyrosinase of plants and lower animals is not specific, which limits the value of the latter evidence. Fox and Millott (*11*) have shown that melanin derived from black feathers gives, after fusion with potassium hydroxide, a positive Ehrlich test indicating the presence of a pyrrole nucleus. If further experience shows this to be re-

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[*] According to Lea (*10*) some melanins will dissolve in ethylene chlorohydrin, cellosolves, and mixtures of ethyl lactate and benzyl alcohol.

liable it should be of considerable value in distinguishing between melanins and other dark pigments. Unfortunately modern spectroscopic techniques are of little value for identification purposes. Melanins show only general absorption in the ultraviolet and visible regions of the spectrum, with no useful characteristic features, and infrared spectra are equally poor and disappointing.

II. The Initial Stages of Melanogenesis

The first indications of the origin and biogenesis of melanin were coincident with the discovery (*12*) of tyrosinase in certain higher plants and fungi which are prone to blacken when injured and exposed to air. In 1896 Bertrand (*13*) isolated a crystalline substance, tyrosine, from the fungus *Russula nigricans,* and when this was brought into contact with an enzyme extracted from the juice, the solution turned red and finally deposited a black precipitate. Within a short time similar results were obtained with extracts from many other plants and from the tissues of insects and other invertebrates (*2*), and it became apparent that tyrosinase, by its action on tyrosine, might be responsible for much of the dark pigmentation frequently observed in animals and plants. Raper's classic work on the preliminary stages of melanogenesis is discussed below; it will suffice here to note that he found tyrosinase to be an essential requirement for the initial conversion of tyrosine into dopa and then into dopaquinone (see p. 734). As the tyrosine-tyrosinase reaction is the key to melanin formation, a brief review of the properties of this remarkable enzyme is desirable at this stage (for a full account see references 5 and *14*).

Tyrosinase is a copper-protein complex of very wide distribution, occurring almost throughout the phylogenetic scale. Representative sources, taken from a vast literature, have been tabulated by Dawson and Tarpley (*14*), and Yasunobu (*15*), and some further examples are noted in Section III. The enzymes isolated from different sources show qualitative differences, mammalian tyrosinase being relatively specific to L-tyrosine and L-β-(3,4-dihydroxyphenyl)alanine, whereas the enzymes from lower animals, and more particularly from plants, are active toward a wide range of monohydric and dihydric phenols (*15, 16*). The ability to catalyze the oxidation of both mono- and dihydric phenols is characteristic of tyrosinase, and there is now little doubt that both activities derive from a single enzyme. Moreover copper plays an essential role in both activities. The so-called cresolase (phenol *o*-hydroxylase) and catecholase (*o*-dihydric phenol dehydrogenase) activities, shown in the equations on p. 731, each require oxygen; experiments with oxygen-18 have established that the oxygen of the second hydroxyl

$$\text{Phenol} + \tfrac{1}{2}O_2 = o\text{-Dihydric phenol}$$

$$o\text{-Dihydric phenol} + \tfrac{1}{2}O_2 = o\text{-Quinone} + H_2O$$

group is derived from molecular oxygen (*17*). The *o*-quinones formed by catecholase activity are extremely reactive intermediates; that derived from tyrosine undergoes an intramolecular reaction to form an indole derivative which subsequently polymerizes to form melanin; the less specific plant enzymes can give rise to a variety of *o*-quinones leading, by polymerization and interaction with protein, to complex products of completely unknown structure. Although it has never proved possible to isolate the cresolase entity, the catecholase:cresolase ratio can be altered, and in fact this usually occurs during the "purification" process. Unless care is taken, a "purified" tyrosinase preparation may be almost devoid of cresolase activity and becomes a polyphenoloxidase. This is generally ascribed to modification or fragmentation of the protein moiety with consequent changes in the enzyme surface. Mason (*18*) has recently suggested that the entity involved in *o*-hydroxylation (cresolase activity) is a complex formed by combination of a molecule of oxygen with two neighboring cuprous atoms linked to protein. The formation of such a structure would depend upon the relative positions of the copper atoms to one another, and hence cresolase activity would be sensitive to variations in the configuration of the protein chain to which the copper atoms are attached.

The tyrosine-tyrosinase reaction is often marked by a time lag before oxidation commences (*19*, *20*). This induction period can be shortened by the addition of catechol or related *o*-dihydroxyphenyl compounds, and in the case of mammalian tyrosinase, dopa is a fairly specific catalyst (*21*). There is no induction period if dopa is the substrate. This peculiarity arises because tyrosinase is only active toward tyrosine in the reduced (cuprous) state. If the enzyme exists in the oxidized form (e.g., in a medium of high redox potential), it will immediately effect the oxidation of dopa but remain inert toward tyrosine until activated (i.e., reduced) by the addition of dopa (or catechol, etc.). If the concentration of tyrosinase is low, as in human epidermal melanocytes, the induction period is prolonged. This may partly account for the observations of Bloch (*22*), who found, on incubation of sections of human skin in separate solutions containing dopa, tyrosine, and related compounds, that only the dopa was oxidized to melanin. From this and other evidence, it was concluded that the melanoblasts in human skin contained only a specific "dopa oxidase." It is comparatively difficult to demonstrate the presence of tyrosinase in mammalian tissues, and many years elapsed before Fitzpatrick and co-workers (*23*) established its existence in human epidermis. Many substances inhibit tyrosinase activity (*20*), notably thiol compounds which combine with copper. Inhibitors of this type have been found in human epidermis (*24, 25*) and are probably mainly responsible for the inactivation of tyrosinase in normal unpigmented skin. Flesch (*25*) has shown that the concentration of thiol groups in human epidermis diminishes on exposure to ultraviolet radiation, and it is known from other work (*26*) that tyrosine can be converted into dopa under similar conditions. These photochemical oxidations, probably effected by hydroxyl radicals, are important factors in the formation of melanin which occurs during sun tanning. Oxidation of thiol groups releases the bound copper by removal of the natural inhibitors, and the formation of dopa catalyzes the tyrosine-tyrosinase reaction. The tyrosinase system in human epidermal melanocytes is normally inhibited unless activated by radiation, whereas that in the melanocytes of pigmented human hair is active (*27*); this accounts for the coexistence of black hair and white skin, which is not uncommon.

A synthetic melanin is very easily obtained in the laboratory by the enzymatic oxidation of tyrosine or dopa under physiological conditions, and with care the initial phases of melanogenesis can be observed visually and spectroscopically. The isolation of two of the intermediates almost at the outset of Raper's elegant investigations on the *in vitro* oxidation of tyrosine (*28*) enabled rapid progress to be made, and the oxidative sequence shown on p. 734 was quickly established. The precise conditions under which melanin is formed are extremely important, and variations, even minor ones, can have a marked effect on the reaction sequence and on the structure and properties of the final product. The importance of pH control was recognized by Raper, and most recent work on the enzymatic oxidation of tyrosine has been done at pH 6.85. Temperature control is also desirable, and the rate of reaction is influenced by the presence of certain heavy metals (*29*), but the most variable factor, as we have seen, is the enzyme tyrosinase. The enzyme preparations used by different workers have differed in origin and consequently in activity, both absolutely and in their cresolase: catecholase ratio; this is probably largely responsible for the conflicting results obtained in different laboratories and the inability of one group to repeat the experiments of another. As melanogenesis is a multistage process involving fast and slow reactions, some enzyme catalyzed, others requiring only oxygen, variations in the activity and in the amount of tyrosinase used are bound to affect the results, and it is desirable that the activities of each preparation be estimated before use. This particular difficulty is avoided when melanin is prepared by autoxidation (although this method is divorced from natural melanogenesis), but here there are other pitfalls for the unwary. Swan and co-workers (*30*) have found recently that different melanins are obtained by autoxidation of β-(3,4-dihydroxyphenyl)ethylamine at pH 8 according to whether oxygen or air is bubbled through the solution! This shows very clearly how a seemingly trivial alteration of the conditions can affect the melanization process.

The oxidation of tyrosine and dopa to melanin is accompanied by definite color changes, the first being the appearance of a red color (max. 475 mμ) which is followed by a colorless stage. By effecting the oxidation of tyrosine with molecular oxygen and tyrosinase at pH 6–6.5, and then allowing the red solution to decolorize in the absence of oxygen, Raper (*28*) was able to isolate 5,6-dihydroxyindole* together with a trace 5,6-dihydroxyindole-2-carboxylic acid.* (The latter is the major product if the red solution is allowed to decolorize in an atmosphere of sulfur dioxide.) On exposure to air, especially in alkaline solution, 5,6-dihydroxyindole rapidly darkens and deposits a black precipitate, but the autoxidation of 5,6-dihydroxyindole-2-carboxylic acid is much slower and does not give a melanin. In later work (*31*), as already mentioned, it was found that tyrosinase was required only for the formation of dopaquinone (see Raper's scheme), the other reactions proceeding spontaneously, although the rate increases if the enzyme is present. The identification of these indole derivatives led Raper to formulate the red compound (dopachrome†) as 2,3-dihydroindole-5,6-quinone-2-carboxylic acid, and the preceding steps as shown in the scheme. The formation of dopachrome from dopaquinone is a normal quinone-amine addition reaction, but the final base-catalyzed rearrangement of dopachrome is a special feature of such *o*-quinonoid structures (aminochromes). The corresponding *p*-quinones are stable to base (*33a*). In the case of dopachrome the rearrangement is accompanied by decarboxylation except under acid conditions (*34*). The decarboxylative rearrangement of dopachrome is catalyzed also by zinc ions (*35*), and it is noteworthy that the choroid of the vertebrate eye, which is heavily melanized, contains a remarkably high concentration of zinc, greatly exceeding that in non-pigmented tissues (*6*). Its physiological function, however, is not clear.

On oxidation of 5,6-dihydroxyindole in the presence of tyrosinase at pH 5.6, Mason (*34*) observed the appearance of a purple pigment (max. 540 mμ) spectroscopically identical with that formed in the second phase of the enzymatic oxidation of dopa. This was considered to be

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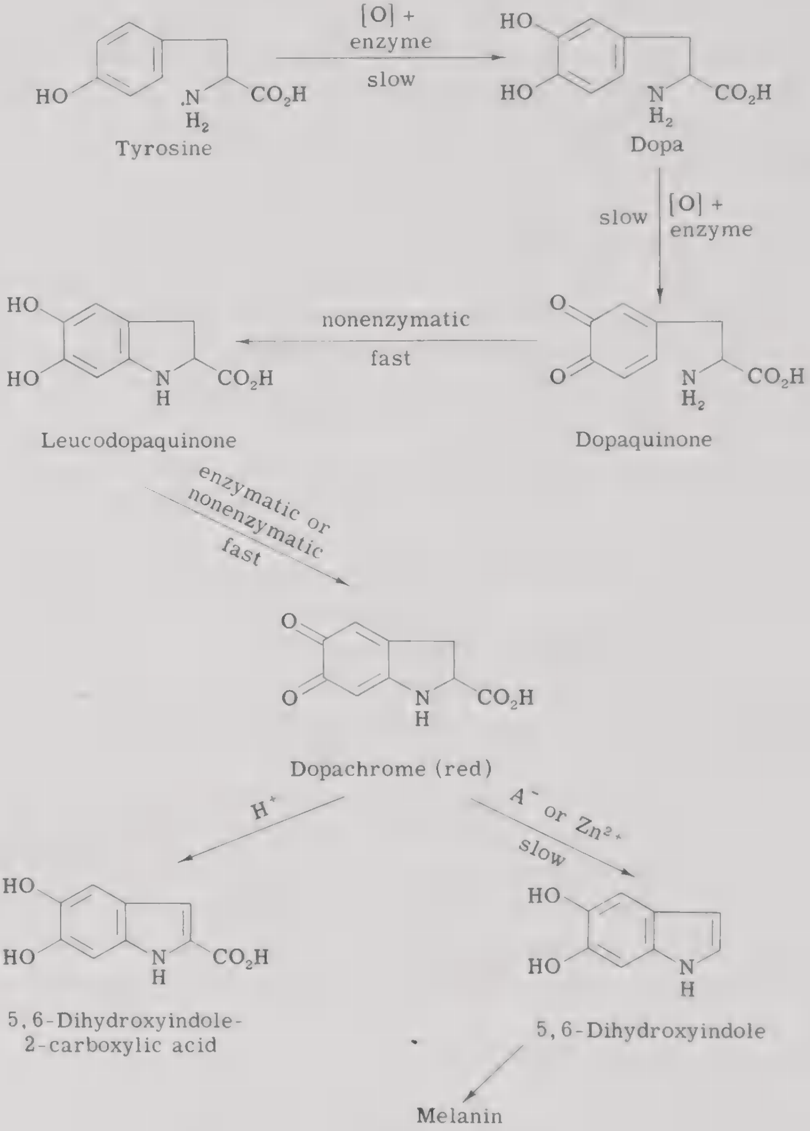
* As its dimethyl ether.

† Dopachrome must not be confused with hallachrome (*32*), the red pigment of *Halla parthenopeia*. The two compounds are quite different (*33*). (See p. 677.)

Raper's Scheme of Melanogenesis



indole-5,6-quinone [first suggested by Raper (*36*) as a probable intermediate], but Robertson and co-workers (*37*), who confirmed Mason's observations, pointed out that the light absorption of the purple pigment (melanochrome) was similar to that of the indolylbenzoquinones prepared by Cromartie and Harley-Mason (see below), and they regarded melanochrome as a polyindolequinone comprising a small number of linked indolequinone units. In studying the autoxidation* of various

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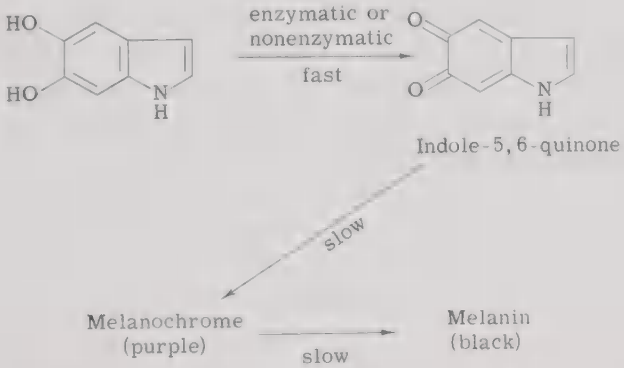
* Enzymatic oxidation was not used as preliminary experiments showed that tyrosinase had a negligible effect on the visible phenomena of the reaction.

5,6-dihydroxyindoles at pH 6.85 (see Table II), Cromartie and Harley-Mason (*38*) found that in most cases the reaction was too rapid to permit spectroscopic identification of intermediates, but 2,3-dimethyl-5,6-dihydroxyindole formed a comparatively stable red substance (max. 470 mμ) whose light absorption was similar to that of dopachrome. The same red product is formed when 2,3-dimethyl-5,6-dihydroxyindole is oxidized with silver oxide in methanol, or with ferricyanide in aqueous solution (this is reversible below pH 5), and it appears to be 2,3-dimethylindole-5,6-quinone. [The yellow compound Robertson and coworkers obtained by oxidation of 2,3-dimethyl-5,6-dihydroxyindole with silver oxide in acetone is possibly a Diels-Alder dimer (*38, 39*) or a quinone-acetone adduct (*40*)]. As 2,3-dimethylindole-4,7-quinone also shows an absorption peak at 470 mμ (*41*), the purple pigment can hardly be monomeric indole-5,6-quinone; the suggestion that it is a low polymer seems more reasonable.* The later stages of melanogenesis are therefore summarized as shown.



III. The Natural Distribution of Melanins

A. PLANT MELANINS

The formation of dark-brown or black pigments during the normal development of plants is a well-known phenomenon commonly observed as markings on petals and leaves, in the spores and hyphae of the higher fungi, in senescent leaves and seedpods, and in the dead cells of bark, seedcoats, and pericarps. Furthermore, many plant tissues darken rapidly on injury. It is likely that many of these pigments arise by the enzymatic oxidation of phenols to quinones which are subsequently converted into complex products by polymerization and interaction with protein. Such reactions can be demonstrated *in vitro* (*42*) and are akin to those that occur in the formation of melanins, but the products are not melanins. Very few of the dark pigments formed by plants have been investigated, and unfortunately the scanty evidence which has been obtained by direct examination of the natural coloring matters relates chiefly to nonmelanic pigments. The Japanese lacs, studied by Bertrand (*43*), Majima (*44*), and later workers, are the oxidation products of polyphenols exuded by many Anacardiaceae (chiefly *Rhus* spp.), and the black phytomelanes elaborated in the fruits of certain Compositae are also nitrogen free (*45*). In passing, it may be noted that the essential oils of many Compositae contain polyacetylenic compounds which occur also in higher fungi (*46*). These highly unsaturated substances frequently are unstable and blacken on exposure to light. This is not known to occur *in vivo*, but conceivably it could. The glucoside, aucubin (I), is responsible for the blackening of the leaves, fruits, and other parts of the Japanese variegated laurel *Aucuba japonica* (*47*) and is found in the leaves and seeds of many other plants (*48*, *49*). The black material is an oxidation product of the aglycon aucubigenin (*50*), which is liberated by the action of a β-glucosidase present in the tissues. Many of the dark pigments in plants may be of

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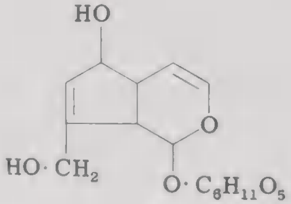
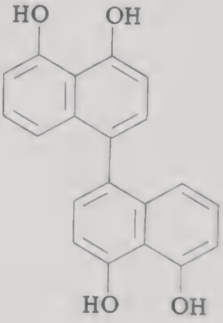
* This view is supported by the recent spectroscopic studies of Bu'Lock (*41a*). The dimeric stage in the tyrosinase oxidation of 5,6-dihydroxyindole has been detected as a transient purple pigment (max. 530 mμ), probably a 5,6-dihydroxyindolylindole-5,6-quinone, preceded by its reduced form which has a characteristic fluorescence. Subsequent polymerization converts the dimer into oligomers with absorption maxima around 540 mμ, and finally into melanin.



(I)



(II)

this type (*49*), but clearly they are not melanins. Another example of a black, nonnitrogenous pigment is seen in the fungus *Daldinia concentrica*. Allport and Bu'Lock (*51*) have shown that the chromogen is the polyphenol (II), which is oxidized enzymatically to 4,9-dihydroxyperylene-3,10-quinone and a black polymer.

Although it is evident that none of the pigments cited so far are melanins in the accepted sense, it is more difficult to say with certainty which plant pigments *are* melanins since no "presumed melanin" has yet been isolated and subjected to adequate chemical examination. We must therefore rely on the inadequate criterion that a plant melanin is a dark pigment formed during normal ontogeny, or following upon injury, in tissues known to contain the tyrosine-tyrosinase system. The situation, in short, has not significantly changed since Bertrand (*13*) first obtained tyrosine and tyrosinase from dahlia tubers and other plants in 1896, except that dopa and closely related compounds are recognized as alternative substrates. Both tyrosine and dopa have been detected in the broom *Sarothamnus scoparius* (*52*), together with tyramine, hydroxytyramine, and epinine, all of which yield melanins on oxidation. It is noteworthy that the concentration of all these compounds is greatest in the fall when melanization is most apparent. Griffiths (*53*) has demonstrated recently that the blackening of bananas, which occurs frequently on storage, is due to enzymatic oxidation of hydroxytyramine present in the skin and pulp. A number of bacteria (e.g., *Bacillus niger*) produce black pigments. The melanin formed by one of these (*B. salmonicida* or a close relative) was isolated by Lloyd and Johnston (*54*) from laboratory cultures as a purple-black powder. It formed a dark brown solution in aqueous sodium hydroxide which was reprecipitated by acid, and contained nitrogen. The presence of tyrosinase was also established, and blackening was shown to be dependent upon access to oxygen and a substrate containing tyrosine. Little comparable work appears to have been done on the presumed melanins of fungi or higher plants, although the blackening of potato tubers, a matter of some economic importance, has received attention. Both tyrosine and tyrosinase are present in potatoes (*55*), and the crude pigment isolated from black spot discolorations by Van Middelem and coworkers (*56*) was spectroscopically similar to synthetic melanin prepared by the tyrosine-tyrosinase reaction. Much more work on the isolation and purification of plant melanins is required, as only in this way can they be properly identified and compared with the animal pigments.

A hint as to the possible course of melanogenesis is given by the co-occurrence of tyrosine and dopa in a number of melanin-producing plants [e.g., *Vicia faba* (*57*)] and by the observation that the blackening of plant tissues sometimes proceeds via a red phase, as, for example, when potato slices are ground up or exposed to chloroform vapor. These facts are consistent with Raper's scheme of melanogenesis, which could probably be established by detailed investigation.

Tyrosine (*48, 58*) and tyrosinase both appear to be widely distributed throughout the plant kingdom, but the occurrence of melanins is much more restricted. Moreover black pigmentation is often highly localized in individual plants in contrast to the more general distribution of the enzyme (*57*). This may be attributed either to the presence of inhibitors (ascorbic acid is of common occurrence) or to circumstances which, under normal conditions, prevent the enzyme and substrate having simultaneous access to oxygen. This situation breaks down on disruption of the cell structure and melanin formation can then proceed; alternatively injury to the tissues may result in oxidation of the inhibitor with consequent loss of inhibitory capacity. An example of ascorbic acid inhibition is seen in *Stizolobium hassjoo;* blackening takes place most rapidly in the younger leaves, which have the lowest concentration of ascorbic acid (*59*). In a study of melanin formation by a black mutant of the fungus *Neurospora crassa,* Schaeffer (*60*) was able to correlate increased pigmentation with increased tyrosinase activity, but he was unable to decide whether this was due to the presence of a larger amount of enzyme or to a partial block in the synthesis of a tyrosinase inhibitor. The inhibitor in this case may be a thiol, as Horowitz and Shen (*61*) found that tyrosinase activity in *Neurospora* was dependent upon sulfur nutrition.

B. Animal Melanins

The melanins found in the animal kingdom (*62, 63*) are of much greater biological interest than the plant melanins. As mentioned earlier, mammalian melanins in particular have been the subject of numerous investigations, and a number have been isolated and subjected to chemical examination. The situation is less satisfactory among the lower animals where there is much reliance on inadequate histochemical tests for purposes of identification, and much of the older work does little more than record the existence of black pigments, which, as we have seen, are not necessarily melanins. In more modern work a search is usually made for an accompanying tyrosinase system, although this too, is of uncertain value, as these enzymes are not specific. Nevertheless it seems likely that the innumerable shades of black and brown frequently observed in vertebrates and invertebrates are produced mainly by melanins occurring in different states of oxidation and aggregation, accompanied sometimes by other pigments. As may be seen from the following examples, these pigments are of general occurrence in many phyla.

The most convincing description of melanin in a primitive animal is that of Fox and Pantin (*64*) on the plumose anemone *Metridium senile.* Colored varieties were found to contain a black granular pigment in the endoderm which responded to the usual tests and a more diffuse brown melanin in the ectoderm; both white and colored animals possess a complete tyrosinase system. Tyrosinase is present in the tissue fluids of sponges (e.g., *Suberites domuncula, Tethya lyncurium*) (*65*), but its relevance to the black pigments occasionally observed in the Porifera is unknown (cf. *65a*). It is of interest that a magenta chromoprotein found in the jellyfish *Pelagia noctiluca* contains a brown chromogen which appears to be an indole derivative (*11*). However, no tyrosinase could be detected, and its relationship to melanin, if any, is doubtful. That the flatworms *Planaria lugubris* and *Polycelis nigra* vary in color from white to black is "due to different depositions of melanin in the skin" (*66*). In support of this assertion it was pointed out (*67*) that some of the animals were pink, the color being attributed to one of the Raper quinones (p. 734). This seems improbable, but spectroscopic reinvestigation would be of interest. Dark pigmentation is not uncommon among echinoderms, and Millott and Jacobson have shown, by chemical and histochemical tests, that the characteristic black granular pigment in the large epidermal melanophores of *Diadema antillarum* (*68*) and *Thyone briareus* (*69*) is probably melanin. The amebocytes of these animals are associated with a phenolase complex, and if the coelomic fluid is exposed to air, clots of aggregated amebocytes are thrown down which rapidly become red and then gradually darken to brown or black, producing a pigment very similar to that in the skin. A similar enzyme system is present in the amebocytes of the holothurioid echinoderm *Holothuria forskali,* which contains an abundance of melanin in the body wall (*70*). Integumentary black pigment is frequently visible in gastropods and bivalves, and a copious supply is present in the specialized anal gland or ink sac of cephalopod mollusks. The sac of *Sepia officinalis* contains up to 10 ml. of an intensely black suspension of melanin granules. Tyrosinase activity in both *Sepia* glands and dried *Sepia* sacs has been reported (*71*), but its association with the pigment granules does not appear to have been demonstrated.

The black pigment in the hypodermis of certain crustaceans (e.g., *Cancer pagurus, Crangon vulgaris*) is associated with tyrosinase and shows the general characteristics of melanin (*72*), and there is good evidence that melanin formation is responsible for the undesirable "black spot" darkening of fresh shrimp (*73*). The blood of many insects, of which tyrosine is a common constituent (*73a*), rapidly darkens on exposure to air; likewise, many pale newly hatched larvae darken in a short time after emergence. Since a tyrosinase system is very widely distributed in insects (*74*), melanin formation is clearly implicated although the pigment is not usually granular. The position is complicated, however, by the presence of protocatechuic acid and other polyphenols, and it has been difficult to disentangle the process of melanization from cuticular darkening due to sclerotisation.* However, recent work has established that the two processes are independent. In experiments with the cuticle of the desert locust (*Schistocerca gregaria*) Malek (*76*) was able to show that blackening could occur unaccompanied by tanning, the onset of sclerotization being delayed until melanization was complete. Furthermore, Dennell (*77*) found that when melanin formation in the larvae of the blowfly (*Calliphora vomitoria*) was completely inhibited by injection of phenylthiourea, puparium hardening still continued normally.† There is much evidence that tyrosinase is distributed generally throughout insect tissues, but the formation of melanic pigment patterns indicates that either the chromogen is more localized or that inhibitors are present in certain areas.

The chordate melanins are frequently prominent in skin, hair, feathers, and scales and occur also in choroid, peritoneum, pia mater, and other membranous tissues in mammals, birds, reptiles, amphibians, and fishes. Melanotic tumors are not uncommon among vertebrates. The presence of tyrosinase has been demonstrated in the melanic tissues of many higher animals (*14*, *20*), and as these pigments are comparatively accessible, a number have been isolated for study. The available evidence (see Section IV) suggests that mammalian melanins are probably all similar in chemical character. Less is known concerning the nature of other vertebrate melanins although their distribution in birds and amphibia has received much attention (albeit mainly by geneticists), and some remarkable patterns of melanization have been described (*62*). Silky fowls for example, are a white-feathered breed, yet their skins are black and melanin is widely distributed throughout the body. The adaptive coloration of axolotls (*Ambystoma mexicanum*), frogs (*Rana temporaria*), and certain fishes (e.g., *Lebistes reticulatus*) arises from their ability to control melanin formation in response to background illumination. In dark surroundings both the number of melanophores and the actual amount of pigment increases, but the opposite effects are produced against a light background. Integumentary melanins are indirectly responsible for many structural (as distinct from pigmentary) colors displayed by animals, notably the Tyndal blues seen in certain skin areas of numerous fishes, reptiles, and mammals and in the feathers of birds. The effect is seen when light is scattered by small particles in a transparent layer of tissue overlying a dark screen of melanin which absorbs the light of longer wavelength. Pigmentary and structural colors are sometimes combined; this can be observed in the green feathers of birds which change to Tyndal blue on extraction of the concomitant yellow carotenoids. Melanin also plays an important background role in the display or iridescent (interference) colors frequently seen, for example, in the skin of reptiles and fishes. The coexistence of black, blue, and green areas in many fishes, together with changeable iridescent colors, is not uncommon, and all these can be attributed, directly or indirectly, to light-absorbing melanophores. [For full discussion of these effects see Fox (*63*)].

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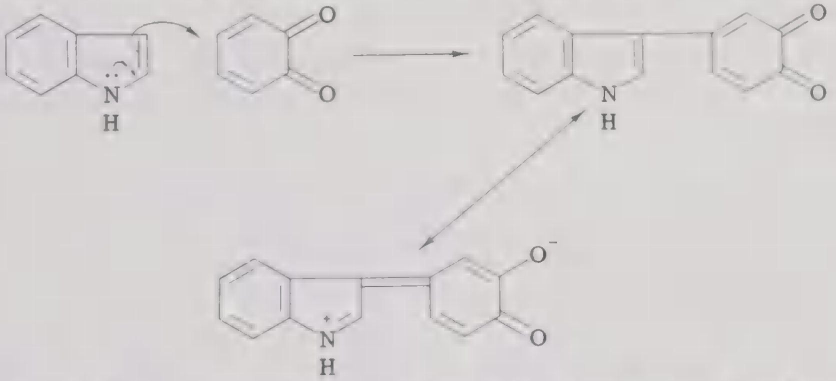
* The hardening of cuticle brought about by quinone tanning, i.e., the cross linking of proteins by reaction with quinones derived from dihydric phenols (*75*). The consequent formation of aminoquinones results in darkening of the cuticle.

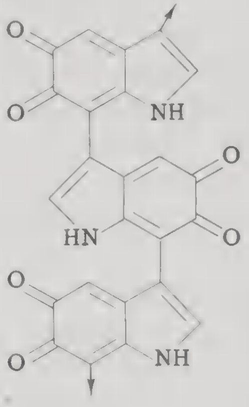
† See also Fuzeau-Braesch (*77a*).

IV. The Structure of Melanins

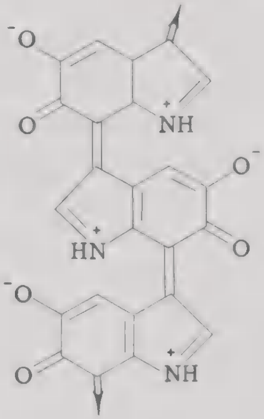
The biogenetic studies of Raper delineated the course of melanogenesis as far as the formation of 5,6-dihydroxyindole, and it has been generally agreed that the final stages involve the oxidative polymerization of indole-5,6-quinone, but the details of this process are difficult to establish. On one view (*78*), the monomer functions both as a quinone and as an indole, the anionoid center in the pyrrole ring of one molecule linking up with a cationoid center in the quinonoid ring of a second molecule, the process being repeated many times. Bu'Lock and Harley-Mason (*79*) showed that such reactions were feasible by model experiments with simple quinones and indoles, *o*-benzoquinones reacting rapidly at room temperature. Reaction normally takes place at position 3 in the indole nucleus, as shown here, although a second condensation with another quinone molecule may occur at an unsubstituted 2-position. If position 3 is blocked, entirely different, colorless, products are formed. These reactions are markedly subject to steric effects and proceed with difficulty, or not at all, if overlapping substituents are present which prevent the formation of a coplanar product. Planar mesomeric structures (see above) make a considerable contribution to the light absorption of the indolylquinones, which are blue violet to almost black in color.



On the basis of these model experiments Bu'Lock and Harley-Mason (79) proposed that melanin is formed by repeated oxidative condensation of indole-5,6-quinone between position 3 of one molecule and position 4 or 7 of another. As the product obtained by 3-4 linkage could not be coplanar and hence, by analogy with the simple indole–quinone reactions, would not form readily, a 3-7 linkage was considered most likely, giving the coplanar structure (III). The extensive conjugation seen in the mesomeric structure (IV) could account for the general light absorption of the polymer. To explain the extreme insolubility of melanin, it was further suggested that occasional condensation might occur at position 2, with position 4 or 7 of another molecule, the cross links leading to the formation of a highly irregular three-dimensional polymer.



(III)



(IV)

TABLE I

Autoxidation of Dopa and Its Methyl Derivatives in Aqueous Buffer (pH 6.85)[a,b]

Position of methyl substituents	Coloration	Time of maximum color (hr.)	Final appearance[c]
2	Red	2.5	Fine black ppt.
5	Violet	2.5	Fine black ppt.
6	Yellow	12	Yellow; no ppt.
None (with enzyme)	Red	0.25	Flocculent black ppt.
None (without enzyme)	None	None	Slight black ppt.

[a] Reproduced (with permission) from R. I. T. Cromartie and J. Harley-Mason, *Biochem. J.* **66,** 713–720 (1957).

[b] Addition of tyrosinase made no difference in the first two cases and only a slight difference in the third case.

[c] ppt. = precipitate.

To test this hypothesis, Cromartie and Harley-Mason (38) autoxidized a series of dopa and 5,6-dihydroxyindole derivatives in which different nuclear positions were blocked by methyl groups. Their results, shown in Tables I and II, were considered to support the view that an unsubstituted 3-position is essential for the formation of a melanin, i.e., a black pigment of high molecular weight, together with a free position at either C-4 or C-7, but it was recognized that positions, 2, 3, 4, and 7 were probably all concerned to some extent. It will be noted (Table II) that both 3-methyl and 4,7-dimethyl-5,6-dihydroxyindole gave highly colored products, but unlike the melanins, they were soluble and were formed much more rapidly. These results are in broad agreement with those of Robertson and co-workers (37) obtained by autoxidation of 5,6-dihydroxyindoles at pH 8, but one discrepancy is perhaps significant. Whereas Cromartie and Harley-Mason obtained a stable red solution by autoxidation of 2,3-dimethyl-5,6-dihydroxyindole at pH 6.85, ascribed to 2,3-dimethylindole-5,6-quinone, at pH 8 Robertson and co-workers obtained a black polymer of considerable molecular weight; because of this they concluded that 4-7 linking of indole-5,6-quinone units is possible.

TABLE II

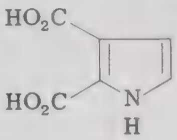
AUTOXIDATION OF METHYLATED 5,6-DIHYDROXYINDOLES IN AQUEOUS BUFFER (pH 6.85)[a,b]

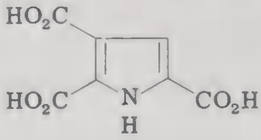
Position of methyl substituents	Times of appearance (min.)			Appearance of precipitate	Solubility of precipitate
	Color	Opalescence	Precipitate		
None	20	30	90	Black, flocculent	Insoluble in pyridine
1	None	90	240	Black, fine	Dispersed in hot pyridine
2	10	30	360	Black, fine	Insoluble in pyridine
3	5	None	30	Blue, flocculent	Soluble in ethanol and alkali
2, 3	10	None	None	None	None
4	15	40	360	Black, fine	Dispersed in pyridine
7	10	20	180	Black, fine	Readily dispersed in pyridine
4, 7	3	None	25	Purple, fine	Extracted by ethyl acetate

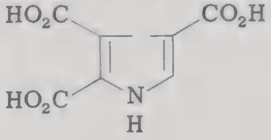
[a] Reproduced (with permission) from R. I. T. Cromartie and J. Harley-Mason, *Biochem. J.* **66**, 713–720 (1957).

[b] The dihydroxyindole (15 μmoles) was dissolved in 100 ml. of buffer (pH 6.85) and oxygen was bubbled through the solution.

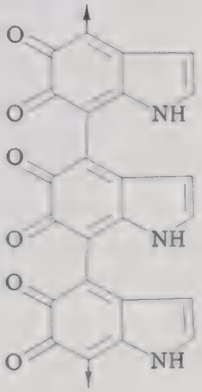
The classic degradative approach to the structure of complex products is notoriously difficult in the case of melanin, but some success has been achieved by Nicolaus and his collaborators (*80*, *81*). Oxidation of synthetic tyrosine melanin with hydrogen peroxide effected a partial breakdown of the structure, yielding an ether-soluble fraction containing two pyrrolecarboxylic acids identified on paper chromatograms as (V) and (VI). This has been confirmed by Binns and Swan (*82*), who


(V)

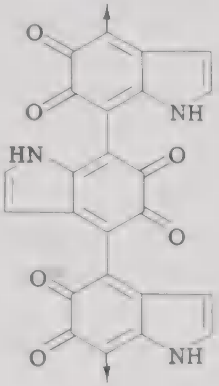

(VI)


(VII)

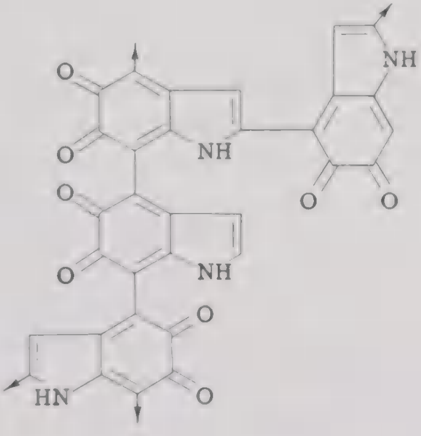
subjected a variety of synthetic melanins (obtained by autoxidation of tyrosine, dopa, β-(3,4-dihydroxyphenyl)ethylamine, and 5,6-dihydroxyindole) to oxidation with hydrogen peroxide. The same two pyrrolic acids were detected (along with four unidentified products in the case of tyrosine melanin), but, as these authors point out, the yields are extremely small, which makes it difficult to assess their importance in regard to the structure of the melanins. The most striking feature of these degradative studies is the failure to detect pyrrole-2,3,4-tricarboxylic acid (VII), which should be formed if tyrosine melanin has the 3-7 linked "backbone" structure (III). Since this acid (VII) can be detected among the degradation products formed when violacein is oxidized under similar conditions (*83*), its absence in the melanin degradations cannot be explained by its formation and subsequent destruction, and would appear to be significant despite the low yields of the other acids. This implies that the formation of melanin does not involve the condensation of the indole-5,6-quinone at position 3 to any appreciable extent, the inter-indole link being mainly at the 4-7 and 2-4 (or 7) positions, in contradiction to the Harley-Mason theory. It will be recalled that Robertson and co-workers drew attention to the possibility of continued 4-7 condensation (see also ref. *84*), and the Ehrlich reaction given by the melanin in black feathers is, perhaps, a pointer in this direction. Indeed it can be argued from the results of Cromartie and Harley-Mason that the 3-position of 5,6-dihydroxyindole is *inessential* for melanin formation. Mason (*42*) points out that 3-methyl-5,6-dihydroxyindole is more susceptible to oxidation than 5,6-dihydroxyindole itself (Table II), and as the products of autoxidation are rapidly removed from solution, the opportunity for extensive polymerization (melanin formation) is lost. Accordingly he suggests, following Nicolaus and Mangoni (*81*), that dopa melanin may have a 4-7 linked "backbone" structure, e.g., (VIII) or (IX), with (X) representing a cross-linked structure. Although these are written in a fully oxidized form, the polymer is actually in a lower state of oxidation. Recent electron spin resonance studies of synthetic and natural melanins by Mason *et al.* (3), show that they all have free radical character, which is attributed to



(VIII)



(IX)

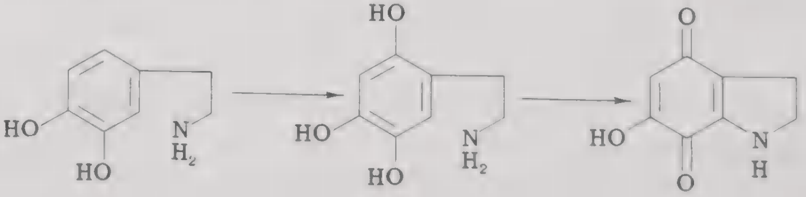


(X)

semiquinonoid structures stabilized by resonance in systems of extended conjugation. Strong support for such a structure comes from the work of Swan and Percival (85) using labeled precursors. Oxidation of β-(3,4-dihydroxyphenyl)-alanine monodeuterated on the β-carbon atom, either with molecular oxygen and mushroom tyrosinase at pH 6.8 or without the enzyme at pH 8, gave a melanin in which most of the deuterium was retained. Had oxidative condensation occurred at position 3 (indole numbering), the deuterium would have been lost.

Against the predominance of 4-7 linked structures may be cited the results of Swan and Wright (*86*) on the autoxidation of β-(3,4-dihydroxyphenyl)ethylamines labeled with carbon-14. The hydrogen peroxide formed during the autoxidation leads subsequently to partial degradation of the polymer with the production of carbon dioxide. It appears that about one indole unit in five is oxidized and approximately 30% of the carbon dioxide originates from each of positions 4, 5, and 6. The difficulty vis-à-vis Mason's structure (VIII) lies in the formation of carbon dioxide from C-4, but whereas the carbon dioxide arising from C-5 and C-6 has been measured experimentally, the figure for C-4 is merely an approximate, hypothetical, value. The difficulty is probably not a serious one, but should be borne in mind as all the synthetic melanins from which pyrrolic acids have been obtained appear to have been prepared under autoxidative conditions.

It is difficult to say how much of the work with model substances has any real bearing on the biogenetic problems. Autoxidative polymerization, which produces *inter alia* hydrogen peroxide and its attendant complications (*85*, *86*), is certainly different from the enzymatic process and may result in quite different structures, and the indole–quinone studies of Bu'Lock and Harley-Mason could be quite irrelevant to natural melanogenesis. More attention should perhaps be paid to the fact that dopa and 5,6-dihydroxyindole are *catechols*. It is possible that all melanins are essentially catechol melanins, the pyrrole rings in tyrosine melanin being perhaps only secondary factors which provide cross linkages and a greater degree of complexity and irregularity. The need for further investigation is evident, still bearing in mind alternative modes of oxidation. Thus Witkop and co-workers (*33a*, *88*) have observed recently that dopamine (XI) can be converted into hydroxydopamine (XII) by the rat, and by oxidation or autoxidation *in vitro*, and this readily undergoes oxidative cyclization in the presence of base to the *p*-quinone (XIII). However, further studies have shown (*85*) that the melanin which can be obtained by oxidation of the trihydroxy compound (XII) is not the same as tyrosine melanin; (XII) is apparently not a primary intermediate in the formation of the latter.

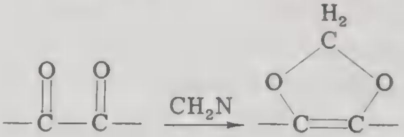


(XI) (XII) (XIII)

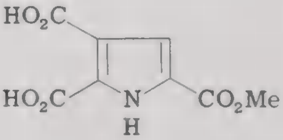
NATURAL MELANINS

The preceding discussion refers only to melanins prepared *in vitro*. We must now consider the more difficult problem of the structure of the natural melanins and their relationship to the synthetic products. Direct comparison of the natural and synthetic materials is unfortunately of little value on account of their intractable nature, the difficulty of purification, and their structural diversity, but there is strong presumptive evidence which indicates that some of the natural melanins are structurally related to synthetic tyrosine melanin. The essential requirements for the formation of tyrosine melanin—the enzyme tyrosinase, tyrosine, or dopa as substrate, and molecular oxygen—are available in many tissues in adequate concentration, and although it cannot be assumed that the formation of melanin *in vivo* invariably follows the Raper pathway, this does seem to be the likely course of events in mammalian melanogenesis, e.g., in melanomas (*20*). In cases of melanotic tumor, substances are excreted in the urine which blacken on exposure to air and deposit melanin. Such a melanogen was isolated by Linnell and Raper (*89*), and although not obtained pure, it appeared to be a simple conjugated derivative of 5,6-dihydroxyindole. Moreover, when 5,6-dihydroxyindole was injected subcutaneously into a rabbit, the urine it excreted for some hours afterward had the typical properties of a melanotic urine, and the melanogen isolated therefrom was very similar, if not identical, to that obtained from human urine. Leonhardi (*90*) and Harley-Mason (*91*) have also detected conjugated 5,6-dihydroxyindoles in melanotic urine, one of which yielded glutamic acid on hydrolysis and was evidently a simple peptide (*92*). It is reasonable to infer from these observations that melanogenesis in melanomas follows the tyrosine → 5,6-dihydroxyindole → melanin pathway. This is also supported by the degradative studies of Nicolaus (*80*) on various mammalian melanins, including those obtained from human and Harding-Passey melanomas, all of which yielded detectable amounts of pyrrole-2,3,5-tricarboxylic acid (VI), previously derived from *Sepia* and dopa melanins by similar treatment with hydrogen peroxide. (It may be noted in passing that the melanins which were oxidized were obtained from hair, skin, the choroid of pig eye, and the two melanomas and had the following range of analytical values: C, 45.28–61.55; H, 4.52–5.97; N, 6.93–9.64%. A simple polyindolequinone structure requires, C, 67.47; H, 2.08; N, 9.65%. Figures for highly purified *Sepia* melanin are given on p. 729.)

Our scanty knowledge of the structure of *Sepia* melanin comes entirely from the work of Nicolaus and his collaborators. On oxidation with hydrogen peroxide it gave small amounts of pyrrole-2,3,5-tricarboxylic acid (isolated as crystalline material) (*93*) and the 2,3-dicarboxylic acid (*81*) and 2,3,4,5-tetracarboxylic acid (*94*), which were detected on paper chromatograms. The latter has not been obtained from synthetic tyrosine melanin. In very recent work (*4*) highly purified *Sepia* melanin has been treated with diazomethane to give a methylated product (OCH_3, 17.43%) which also gave a positive test for methylenedioxy groups. It is postulated that the latter is formed by reaction with an *o*-dicarbonyl (*o*-quinone?) system[*] (XIV), and the methoxyl groups could arise, in part, by methylation of a phenolic (quinol?) structure. Oxidation of the methylated *Sepia* melanin with cold alkaline



(XIV)



(XV)

permanganate failed to give the usual pyrrolic acids, but after hydrolysis of the crude oxidation product, pyrrole-2,4,5-tricarboxylic acid was identified. This indicated that the initial material contained a partial methyl ester of the tribasic acid, probably (XV). Nicolaus *et al.* (*4*) were able also to esterify their *Sepia* melanin by treatment with cold methanolic hydrochloric acid. The methoxyl content (4%) of the product corresponds to the presence of one carboxyl group to every fifth indole unit, assuming a polyindolequinone structure for the polymer. This roughly accords with the findings of Clemo *et al.* (*87*), who showed, by enzymatic oxidation of labeled tyrosine

$$[HOC_6H_4CH_2CH(NH_2)C^{14}O_2H]$$

at pH 8.0, that approximately one-sixth of the carboxyl groups were retained in the polymer. The degradative evidence therefore suggests a parallel between *Sepia* melanin and tyrosine melanin, but of course a polyindolequinone structure is not established for the natural material. Mason and co-workers (*3*) state that there is a general resemblance between the infrared spectra of *Sepia* melanin and dopa melanin, but they were unable to correlate their electron spin resonance spectra although both polymers have free radical character. The origin and biogenesis of *Sepia* melanin is at present obscure, and, as Mason points out (3), no quinone-generating oxidase has been found to be associated with the *Sepia* granules.

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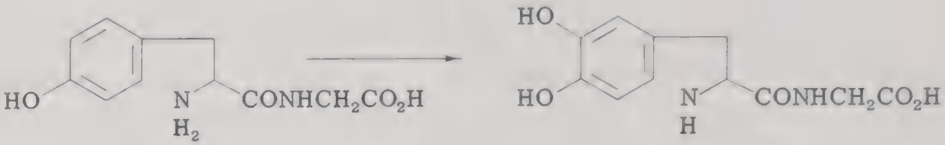
[*] Blitz and Paetzold (*95*) report the formation of methylene ethers by treatment of 9,10-phenanthraquinone, benzil, and similar aromatic compounds with diazomethane, but later workers (*95a*) have shown that these products are α-ketoepoxides.

Natural melanins are generally associated with protein. The protein can be removed, with difficulty, by prolonged hydrolysis with strong acids, but the refractory nature of the pigments makes it difficult to establish how they are linked together. However the weight of evidence (5) indicates that they are chemically bound, i.e., they are melanoproteins. Conjugation could occur either by interaction of the polymer, or one or other of its precursors, with protein, or by oxidation of a protein, containing a terminal tyrosine residue, to a melanoprotein. In the former case, reaction might be anticipated between one of the intermediary quinones in the Raper pathway and free amino or thiol groups in the protein. This has been tested (*42*, see also *96*) by enzymatic oxidation of dopa, and of 5,6-dihydroxyindole and its 2-carboxylic acid, in the presence of simple amines, thiols, and amino acids and peptides containing thiol groups. Reaction was observed (spectroscopically) only when 5,6-dihydroxyindole was oxidized in the presence of thiols, e.g., glutathione. This reflects the enhanced reactivity of thiols normally observed in quinone addition reactions and indicated that the "normal" course of melanogenesis may be deflected in the presence of protein, leading to the formation of sulfur-bound melanoprotein with consequent irregularity in the melanin structure. Greenstein *et al.* (*97*) have concluded also that melanoproteins are conjugated via sulfur bonds, basing the conclusion on their observation that the melanin obtained by pancreatic digestion of the melanoprotein of mouse melanomas contains a higher proportion of sulfur than the parent chromoprotein. It is probable therefore that when melanogenesis occurs in the presence of protein, a sulfur-linked melanoprotein will result. This may explain why sulfur-free melanin is so difficult to obtain. The position of the sulfur bond is not known: attachment to C-4 has been suggested (*98*) and seems likely but is not the only possibility.

There is no direct evidence for the alternative mode of conjugation through a peptide linkage, but the possibility has been demonstrated by *in vitro* experiments. Tyrosyl groups in proteins are undoubtedly oxidized by tyrosinase (*99*), but more precise results can be obtained by using simple peptides. Bu'Lock and Harley-Mason (*79*) have shown that enzymatic oxidation of tyrosylglycine (XVI) gives a red solution, almost identical spectroscopically with that obtained by similar oxidation of tyrosine; on continued oxidation it became brown but did not deposit pigment. When kept under nitrogen, the red solution slowly decolorized, apparently forming a dihydroxyindole peptide (XVII) (as



(XVI) (XVII)

judged by color reactions), which, like 5,6-dihydroxyindole-2-carboxylic acid, does not form a melanin but might form an end group by copolymerization with indole-5,6-quinone derived from unbound tyrosine. Similar results have been obtained recently by Yasunobu and associates (*100*) in a spectroscopic study of the enzymatic oxidation of (XVI) and other *N*-terminal tyrosine peptides. A typical dopachrome absorption curve was observed in the initial stages of the reaction, the final spectrum (after 60 minutes) showing a close resemblance to that of 5,6-dihydroxyindole-2-carboxylic acid. (It is of interest that the *C*-terminal and internal tyrosine peptides, which cannot form dopachrome derivatives, showed dopaquinone absorption patterns.) The formation of the pyrrolic acid methyl ester (XV) on degradation of methylated *Sepia* melanin is consonant with the incorporation of carboxyl groups during melanogenesis (referred to above), either as such or as peptide groups. Furthermore, the degradation of *Sepia* melanin with hydrogen peroxide gives a higher yield of pyrrole-2,3,5-tricarboxylic acid than is obtained from synthetic tyrosine melanin; this is accounted for if *Sepia* melanoprotein is conjugated through peptide linkages as indicated by the model experiments.

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CHAPTER 14

Flavonoid Compounds

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I. Survey of Naturally Occurring Compounds and Their Biosynthesis

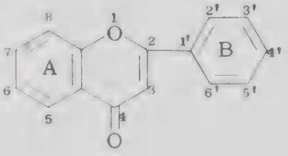
A. Introduction

The main objective of comparative biochemistry must be to throw light on the relationship between different organisms by way of a study of their chemical constituents and the transformations these undergo in the cells and organs of the body or plant. With such an objective in view it will be obvious that chemical substances or biological reactions that play a primary role in normal metabolism and are therefore more or less common to all organisms will be of less consequence in comparative biochemistry than the so-called secondary products of metabolism.

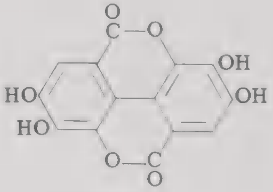
The plant kingdom as a whole possesses a bewildering diversity of such secondary chemical constituents and a corresponding obscurity in the biological reactions underlying their synthesis and function. It is not surprising to find, therefore, that the knowledge which has been gained so far regarding the secondary chemical constituents has been of more value to taxonomy than to physiology. Nevertheless it is always in mind that the presence of any substance is a reflection of the existence of metabolic processes responsible for its formation; and, therefore, its presence is revealing in regard to the physiological processes peculiar to the organism in which it occurs.

Taxonomically the most valuable substances are those that are relatively stable end products of metabolism; these are, as a class, numerous, distinctive, and diverse in their basic structures and are widely and selectively distributed throughout the major taxonomic groups. Examples of such classes of substances are the alkaloids (*1*), the water-soluble plant pigments (*2*), and the terpenoid compounds (*3*).

Of all such secondary substances, by far the greatest diversity in structure is shown by those compounds that contain phenolic groups. Approximately 36% of the naturally occurring compounds of plants listed in Karrer's recently published book (*4*) contain phenolic hydroxyl groups, and of these about one-third are flavonoid compounds; that is, they are anthocyanins, catechins, flavanones, and flavones, which are characterized by possessing the heterocyclic configuration of flavone itself [2-phenylchromone (I)].



(I)



(II)

The flavonoid compounds can also be regarded as C_6–C_3–C_6 compounds, in which each C_6 portion is a benzene ring, and in which the variation in the state of oxidation of the connecting C_3 portion determines the properties and class of the flavonoid compound. Both aromatic rings of most of the flavonoid compounds are substituted with phenolic hydroxyl groups, usually arranged in restricted patterns depending on their biosynthetic origin. In many cases also, certain of these hydroxyl groups are methylated or substituted by sugar residues to give rise to glycosides. Again there is a general restriction as to the phenolic groups so affected.

The designation of flavonoid compounds by C_6–C_3–C_6 brings out the close relationship that exists between them and the cinnamic acids and nearly related coumarins which have a C_6–C_3 structure. In this review both classes will be considered as being covered by the title since together they are more widely distributed than any other class of phenolic substances found in plants. Some attention will be paid to other phenolic substances which are closely related biosynthetically, or are of importance taxonomically, in order that the diversity in over-all structure resulting from the linking of various common building stones may be illustrated. In general these will include examples of the types of compounds covered by Geissman and Hinreiner in their notable review (*5*).

Before surveying in detail the various types of flavonoid and related compounds found in plants, it will be profitable to outline briefly the methods currently used for their analysis (*6, 7*).

In recent years the development of modern separation techniques, especially those based on paper chromatography (*8*), coupled with the extended use of absorption spectrophotometry (*9*), has enabled biochemists readily to separate flavonoid compounds from the complex mixtures obtained in plant extracts and to determine their structure without recourse to isolation in the chemical sense.

Thus extensive surveys have been made of the occurrence of the most commonly occurring flavonoid compounds and cinnamic acids in extracts of higher plants, their identification being based on their behavior and color reactions on paper chromatograms (*2*). Likewise a number of recent papers (e.g., *10*) have dealt with the variation in flower color in phenotypes of single species due to differences in flavonoid constitution, the compounds being separated by paper chromatography and their structure determined by a combination of spectral and microchemical techniques.

In general, plant organs are extracted with aqueous methanol or ethanol, and the individual compounds are isolated from the concentrated extract and purified by preparative chromatography on thick paper or other suitable absorbent. The spectra in solutions of varying pH and in the presence of various chelating ions, the chromatographic behavior, and the color reactions of each substance are then determined and compared with those of known compounds. The individual compounds are then subjected in both a qualitative and quantitative fashion to treatment with mineral acid, which hydrolyzes glycosidic links, and to exhaustive methylation followed by acid hydrolysis in order to determine to which hydroxyl group (or groups) sugars, when present, are attached. After both treatments the products are examined as described above, and from such data the structure can usually be determined.

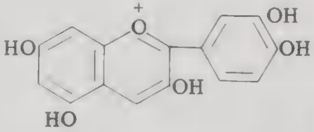
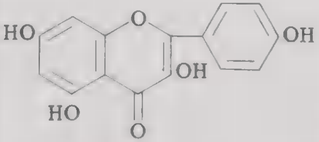
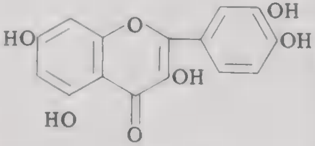
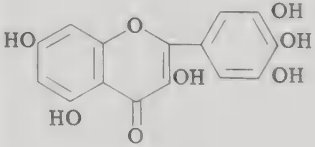
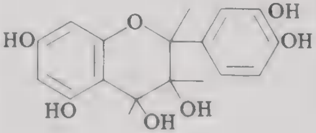
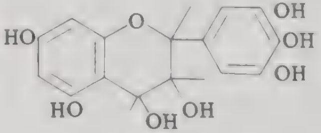
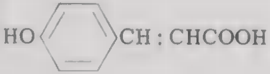
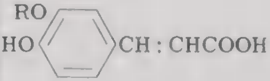
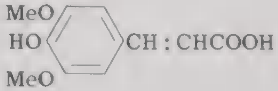
B. SURVEY OF THE VARIOUS CLASSES OF NATURALLY OCCURRING FLAVONOID COMPOUNDS

1. *The Common Classes*

Only a very few of the many natural flavonoid compounds can be regarded as commonly occurring in higher plants (*2, 11, 12*). Ignoring

TABLE I

The Common Flavonoid Constituents of Plants

Class	State of oxidation of B ring		
	Monohydroxy	Dihydroxy	Trihydroxy
Anthocyanidin		 Cyanidin	
Flavonols	 Kaempferol	 Quercetin	 Myricetin
Leucoanthocyanins[a]		 Leucocyanidin	 Leucodelphinidin
Cinnamic acids	 *p*-Coumaric acid	 R = H, Caffeic acid R = CH_3, Ferulic acid	 Sinapic acid

[a] The monomeric flavan-3, 4 diol structure is shown, the names given are trivial.

at present variations based on glycosidation or ester formation, these are the anthocyanidins, of which cyanidin is the most common; the flavonols kaempferol, quercetin, and myricetin; the leucoanthocyanins leucocyanidin and leucodelphinidin; and the cinnamic acids *p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid. The structures of these compounds are shown in Table I.

The hydroxylation-methoxylation pattern of these compounds is also found in a wide variety of other flavonoids, which, although more limited in their distribution than the compounds shown in Table I over the plant kingdom as a whole, are sufficiently common as representatives of a class to be considered together with them (Table II).

It can be plainly seen that the range of structural variation in the known compounds of the flavonoid type is associated primarily with the variation in the state of oxidation of the C_3 part of the molecule. This increases from the most highly reduced catechins to the most highly oxidized flavonols, as shown in Table II. It is also important to note that the pattern of hydroxylation of the A ring (i.e., the benzene ring fused to the heterocyclic portion of the molecule) is either that of phloroglucinol or, less often, that of resorcinol, and that in general the hydroxyl groups of this ring are unmethylated. With rare exceptions the B ring of the flavonoid compounds [the 2-phenyl ring of flavone (I)] contains one, two or three hydroxyl groups; the first substituted in the *para* position to the point of attachment of the ring to the rest of the molecule, with subsequent groups substituted *ortho* or *vicinal* to it.

The hydroxylation pattern of the C_6–C_3 compounds, the cinnamic acids and coumarins, can be seen to resemble that of the B ring in the flavonoids, and the significance of this similarity will be brought out later in the discussion on biosynthesis (page 778).

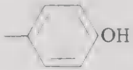
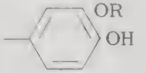
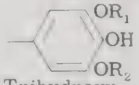
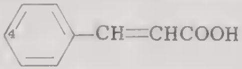
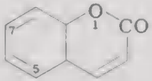
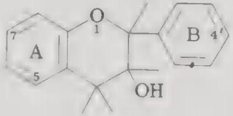
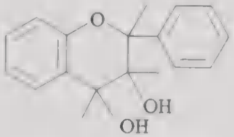
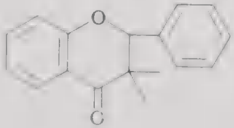
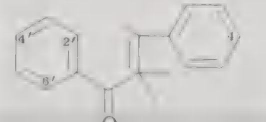
Some restriction on the position of ether formation may also be observed. Thus, in general, the *para*-hydroxyl group in the B ring of flavonoid compounds and the equivalent group in the cinnamic acids and coumarins is unsubstituted, whereas hydroxyl groups *ortho* to it are often methylated, especially in the case of trihydroxy substitution. So far no catechins or leucoanthocyanins of proved structure have been found with methylated hydroxyl groups.

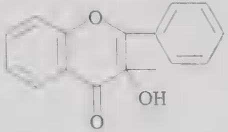
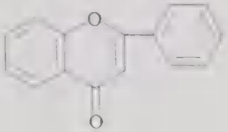
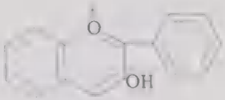
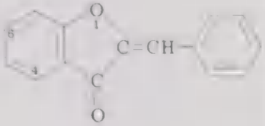
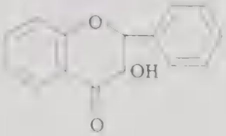
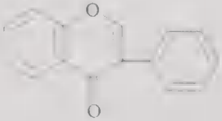
The vicinal trihydroxycinnamic acid has not been isolated from natural sources. In such plants as, on the grounds of analogy, it might be expected to occur, the trihydroxy acid appears to be replaced by hexahydroxydiphenic acid [always isolated as its dilactone ellagic acid (II)], which can be regarded as a dimer of gallic acid (trihydroxybenzoic acid).

With the exception of the catechins and leucoanthocyanins, flavonoid

TABLE II

The Principal Naturally Occurring Flavonoid and Related Compounds

Class of compound	Basic skeleton	State of oxidation of C_3 portion	A ring[a]	Aromatic or B ring		
				 Monohydroxy	 Dihydroxy	 Trihydroxy
Cinnamic acids		$-CH_2 \cdot CHOH \cdot COOH$	None	*p*-Coumaric acid	R=H, Caffeic acid R=Me, Ferulic acid	$R_{1,2}$ = Me, Sinapic acid
Coumarins		$-CH_2 \cdot CHOH \cdot COOH$	None	Umbelliferone (7-OH)	R=H, Esculetin (6, 7-di-OH) R=Me, Scopoletin (6-OMe-7-OH)	R_1 = Me, R_2 = H, Fraxetin (6-OMe-7, 8-OH) $R_{1,2}$ = Me, Isofraxidin
Catechins		$-CH_2 \cdot CHOH \cdot CHOH$	P	Epiafzelechin	R=H, Catechin and epicatechin (diasteroisomers)	$R_{1,2}$ = H, Gallocatechin and epigallocatechin (diasteroisomers)
Leucoanthocyanins		$-CHOH \cdot CHOH \cdot CHOH$	R	Guibourtacacidin	R=H, Gleditsin, mollisacacidin	$R_{1,2}$ = H, Leucorobinetinidin
			P	Leucopelargonidin	R=H, Leucocyanidin	$R_{1,2}$ = H, Leucodelphinidin
Flavanones		$-COCH_2 \cdot CHOH-$	P	Liquiretigenin	R=H, Butin	
			R	Naringenin	R=H, Eriodictyol R=Me, Homoeriodictyol	
Chalkones		$-COCH_2 \cdot CHOH-$	R	2′, 4, 4′-Trihydroxychalkone	Butein	

Flavanonols		–CO·CHOH·CHOH–	R		R=H, Fustin	$R_{1,2}$=H, Dihydrorobinetin
			P	Aromodendrin	R=H, Taxifolin	$R_{1,2}$=H, Ampelopsin
Flavones		CO·CHOH·CHOH	P	Apigenin	R=H, Luteolin R=Me, Chrysoeriol	$R_{1,2}$=Me, Tricin
Anthocyanidins		–CHOH·CHOH·CO–	P	Pelargonidin	R=H, Cyanidin R=Me, Peonidin	$R_{1,2}$=H, Delphinidin $R_{1,2}$=Me, Malvidin R_1=H, R_2=Me, Petunidin
Aurones		–CO·CO·CH_2–	R		R=H, Sulfuretin	
		or				
		CO·CHOH·CHOH	P		R=H, Aureusidin	
Flavonols			R	3,7,4′-Trihydroxy-flavone	R=H, Fisetin	$R_{1,2}$= H, Robinetin
			P	Kaempferol	Quercetin	Myricetin
Isoflavones		–CO·CHOH·CHOH–	P	Genistein	Orobol	

(a) R=resorcinol; P=phloroglucinol pattern of hydroxylation.

TABLE III

Examples of Naturally Occurring Glycosides

Aglycons	Class	Sugar and position of substitution			
		7- or 3-Glucoside	3-Rhamnoside	7- or 3-Rhamnoglucoside	3, 5-Diglucoside
Umbelliferone	Coumarin	Skimmin [a]	–	–	–
Esculetin	Coumarin	Cichoriin [a]	–	–	–
Naringenin	Flavanone	Prunin [a]	–	Naringin [a]	–
Hesperetin	Flavanone	Persicoside [a]	–	Hesperidin [a]	–
Apigenin	Flavone	Cosmosiin [a]	–	Rhoifolin [a]	–
Kaempferol	Flavonol	Astragalin	Afzelin	Nicotiflorin	–
Quercetin	Flavonol	Quercimeritrin [a]	Quercitrin	Rutin	–
		Isoquercitrin	–	–	–
Myricetin	Flavonol	Cannabiscitrin	Myricitrin	–	–
Pelargonidin	Anthocyanidin	Callistephin	–	–	Pelargonin
Cyanidin	Anthocyanidin	Chrysanthemin	–	Kerracyanin	Cyanin
Delphinidin	Anthocyanidin	Myrtillin-a	–	–	Delphin

(a) The 7-glycoside.

compounds and coumarins usually occur in plants as glycosides in which one or more of the phenolic hydroxyl groups are combined with sugar residue. The sugar-free molecules shown in Table II are termed aglycons. The cinnamic acids occur only exceptionally with the phenolic hydroxyl groups in glycosidic combination; more often they are found as esters, the alcohol moiety being in many cases alicyclic or aliphatic hydroxy acids such as quinic acid, or complex glycosides as in echinacoside (*13*).

In any one plant extract, a single aglycon can give rise to numerous glycosides, often as many as six or seven, differing in the nature and number of the sugar residues involved and the position of their attachment. This can be readily observed when an extract of any plant material is chromatographed on paper. Numerous spots will be found which give the typical flavonoid ultraviolet fluorescence, before and after fuming the paper with ammonia, whereas after treatment with hot mineral acid, a chromatogram of the resulting hydrolyzate will show only a few such spots, sometimes only one.

Glycosides of flavonoid compounds may bear the sugar on any of the available hydroxyl groups, but generally the glycosidic links are restricted to certain positions. The anthocyanins, the glycosides of the anthocyanidins, always have sugar residues attached to the 3-position (monoglycosides with a simple sugar, biosides with a disaccharide) and often to both the 3- and 5-hydroxyl groups (diglycosides). In many flavone and flavanone glycosides, the sugar is located in the 7-position whereas in the flavonols the 3- position is favored. As Simpson has pointed out, these hydroxyl groups are the ones which, under certain conditions, may be most easily selectively methylated (*14*).

D-Glucose, L-rhamnose, and D-galactose occur the most frequently in flavonoid glycosides, with L-arabinose, D-xylose, and a number of rarer sugars occurring less frequently. In biosides, where a disaccharide is linked to one phenolic hydroxyl residue, the most common sugar is rutinose, β-L-rhamnosido-6-D-glucose. A selection of commonly occurring glycosides is given in Table III. It can be seen that most of the glycosides have been given trivial names, usually derived from the plant species from which they were first isolated. This makes the task of the worker searching the literature very difficult, and it is hoped that editors of journals will cease to accept authors' recommendations for new names unless these can be seen to be of advantage to the subject as a whole.

2. *The Less Common Classes*

Besides the common classes of flavonoid compounds given in Table II, there is a large number of other flavonoid compounds whose constitution is more complex. This complexity arises from unusual methylation and hydroxyl-substitution patterns, the formation of ethers with complex alcohols, addition of alkyl groups by C—C links, in some cases leading to the formation of extra rings fused to the molecule. Some examples of these are shown in Table IV. It can be seen from these examples, however, that the basic flavonoid pattern exemplified by the compounds listed in Tables I and II is still maintained.

In the review on the biogenesis of flavonoid compounds mentioned earlier, Geissman and Hinreiner (*5*) considered several other classes of phenolic compounds which, in their opinion, needed to be included in the discussion of the subject. It is not intended here to cover all the examples given in their paper, but merely to show the variation that exists in phenolic compounds that are related to the flavonoids in so far as they may arise from the same biosynthetic route. Examples of such substances are shown in Table V. These range from simple benzene derivatives, such as phenol itself, to the complex polycyclic compounds such as rotenone and brazilin.

As can be seen, many structural features of these compounds are common to the building stones of the flavonoid compounds (p. 771). The obvious chemical differences which exist are important since they indicate where a particular species, or higher group, of plant has retained or elaborated certain primitive features of common biosynthetic pathways and as such obviously can be of great potential taxonomic value.

3. *The Polymers*

The phenolic compounds dealt with so far have all been monomeric compounds. There exist in plants, however, two classes of polymeric substances of phenolic nature, namely tannins and lignin.

a. The Tannins. The plant tannins have been surveyed most recently by White (*15*) and Freudenberg (*16*). White defined tannins as the substances responsible for the ability of various plant extracts to tan leather; since the botanical, agricultural, and food literature is full of references to tannins that have not been tested in this way, it would seem more realistic to define as tannins all naturally occurring substances which have chemical and physical properties akin to those which are capable of making leather. This means that they would be water-soluble phenolic compounds, have molecular weights lying between 500 and 3000, and, besides giving the usual phenolic reactions, have special properties such as the ability to precipitate alkaloids and gelatin and other proteins. It is obvious that such a definition will include molecules which are not tannins in the commercial sense of being

TABLE IV

Some Less Common Flavonoid Compounds

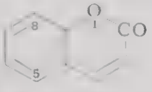
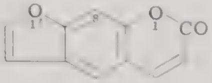
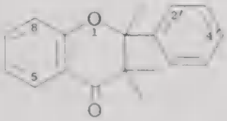
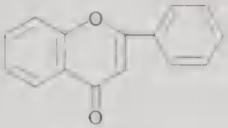
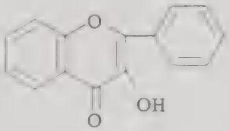
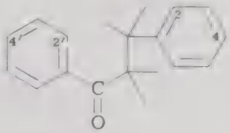
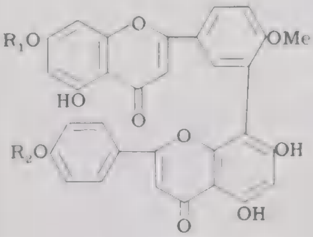
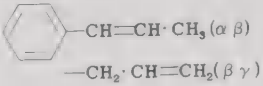
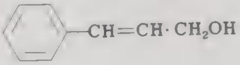
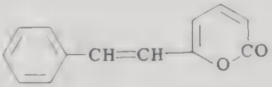
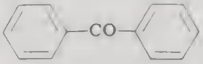
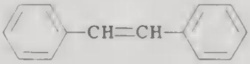
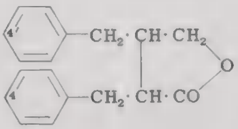
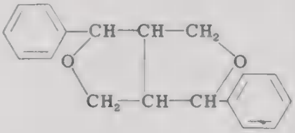
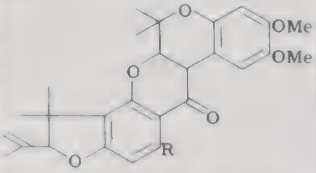
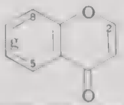
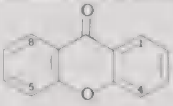
Basic structure	Name	Substitution
 Coumarin	Umbelliprenin	7-Farnesyloxy
	Daphnetin	7, 8-Dihydroxy
	Limettin	5, 7-Dimethoxy
	Osthenol	7-Hydroxy-8-(2-isopentenyl)
 Furocoumarin	Psoralene	
	Bergaptene	5-Methoxy
	Phellopterin	5-Methoxy-8-isopentenyloxy
 Flavanone	Pinocembrin	5-7-Dihydroxy
	Cryptostrobin	5, 7-Dihydroxy-6-methyl
	Methoxybutin	3′, 4′-Trihydroxy-8-methoxy
	Sakuranetin	4′, 5-Dihydroxy-7-methoxy
	Isosakuranetin	5, 7-Dihydroxy-4′-methoxy
 Flavone	Baicalin	5, 6, 7-Trihydroxy
	Wogonin	5, 7-Dihydroxy-8-methoxy
	Strobochrysin	5, 7-Dihydroxy-6-methyl
	Nobiletin	3′, 4′, 5, 6, 7, 8-Hexamethoxy
	Pinnatin	5-Methoxy-3′, 4′-methylenedioxyfuro[3′, 2′g]
	Vitexin	4′, 5, 7-Trihydroxy-8-(α-3, 4-trihydroxy-5-hydroxymethyl) furfuryl
 Flavonol	Galangin	5, 7-Dihydroxy
	Datiscetin	2′, 5, 7-Trihydroxy
	Gossypetin	3, 3′, 4′, 5, 7, 8-Hexahydroxy
	Meliternin	3′, 4′-Methylenedioxy-3, 5, 7, 8-tetramethoxy
	Icaritin	5, -7-Dihydroxy-4′-methoxy-8-(3-hydroxyisoamyl)
 Dihydrochalkone	Phloretin	2′, 4, 4′, 6′-Tetrahydroxy
	Hydroxyphloretin	2′3, 4, 4′6′-Pentahydroxy
 Biflavonyl	Ginketin	R_1 = Me, R_2 = H
	Isoginketin	R_1 = H, R_2 = Me
	Sciadopitysin	R_1 = R_2 = Me

TABLE V

Representatives of Naturally Occurring Phenolic Compounds Related Biosynthetically to the Flavonoids

Class	Structure	Typical representatives
Propenylbenzene		Isoeugenol, 3-methoxy-4-hydroxy (α-β) Eugenol, 3-methoxy-4-hydroxy (β-γ)
Cinnamyl alcohol		Coniferyl alcohol, 3-methoxy-4-hydroxy Sinapyl alcohol, 3,5-dimethoxy-4-hydroxy
Phenylcoumalin		Phenylcoumalin Paracotoin, 3-4-methylenedioxy
Benzophenones		Cotoin, 2,6-dihydroxy-4-methoxy Maclurin, 2,3′,4,4′,6-pentahydroxy
Stilbenes		Pinosylvin, 3,5-dihydroxy Resveratrol, 3,4′,5-trihydroxy
Lignanes	 4′ $CH_2\cdot CH\cdot CH_2$ O 4 $CH_2\cdot CH\cdot CO$ 	*l*-Matairesinol, 3,3′-dimethoxy-4,4′-dihydroxy *l*-Hinokinin, 3,4,-3′,4′-bismethylenedioxy *d*-Pinoresinol, 3,3′-dimethoxy-4,4′-dihydroxy *l*-Eudesmin, 3,3′,4,4′-tetramethoxy
Rotenoids		Rotenone, R = H Sumatrol, R = OH
Chromone		Eugenin, 2-methyl-5-hydroxy-7-methoxy Khellin, 2-methyl-5,8-dimethoxyfuro [3′2′,g]
Xanthones		Euxanthone, 1,7-dihydroxy Gentisin, 1-7-dihydroxy-3-methoxy

economically important in the tanning of hides, but will exclude a large number of substances whose only relationship to the tannins is their capacity to reduce alkaline permanganate or give colors with ferric salts.

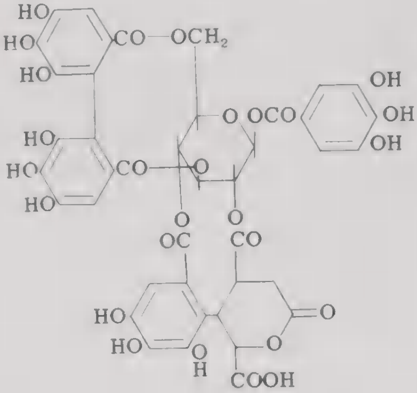
The tannins may be divided into two types, depending on the way in which the various phenolic residues are joined together to achieve the molecular size required. One way is by ester linkage of, for example, gallic acid to a central carbohydrate core, and such molecules may be readily hydrolyzed by acids, bases, or enzymes to yield a carbohydrate moiety and a number of isolable crystalline phenolic compounds. Such tannins are called "hydrolyzable."

Tannins of the second type do not contain carbohydrate as part of their structure, although during isolation they may form strong physical links with polysaccharide material. These substances are called "condensed" tannins.

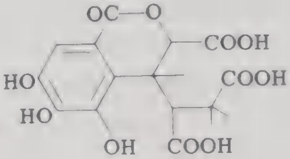
The hydrolyzable tannins include such commercially important extracts as tannic acid, sumach, and myrobalan and can be placed in two groups, depending on whether they yield on hydrolysis gallic acid alone or gallic acid together with ellagic acid (II) and certain other closely related compounds. The structure of all such tannins has not been elucidated with certainty, but members of the first group contain mixed esters of gallic and *m*-digallic acid with glucose or other sugars. Thus the purified fraction of tannic acid, which accounts for 70% of the original commercial extract, appears to have a gallic acid:glucose ratio of 4–5:1 (*17*) as distinct from the earlier accepted ratio of 9–10:1 (*18*). Recent investigations on staghorn sumach tannin (*Rhus typhina*) have shown that the main compound has a tetrasaccharide core of glucose, rhamnose, and arabinose in the ratio 2:1:1, carrying 8 galloyl and 4 *m*-digalloyl groups (*19*).

The chemistry of the ellagic acid-yielding tannins has been more closely studied. One of the compounds from myrobalans and divi-divi extracts, chebulagic acid (*20*), has the structure shown (III), thus yielding on hydrolysis one molecule each of glucose, gallic acid, ellagic acid, and chebulic acid (IV), which is plainly derived from the ellagic acid itself. The chemistry of other compounds is covered in White's authoritative review (*15*).

The structure of the condensed tannins is much less known, and at present there is still some doubt about the actual building stones from which the polymers are elaborated (*15, 21–23*). The majority of workers have accepted that these are in the main flavan-3,4-diols (leucoanthocyanins), possibly with flavan-3-ols (catechins) linked as copolymers, since in many cases monomeric compounds have been isolated along with polymers, whose chemical reactions indicate that they contain the



(III)



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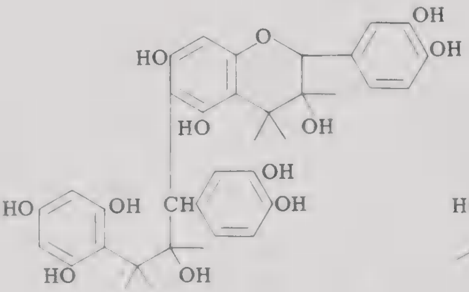

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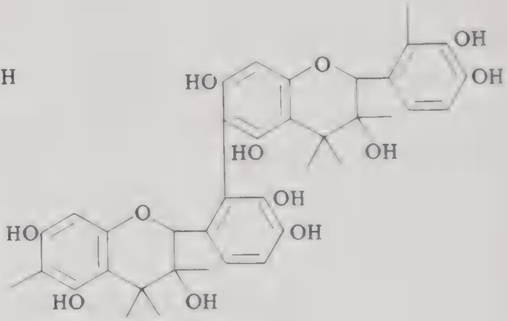

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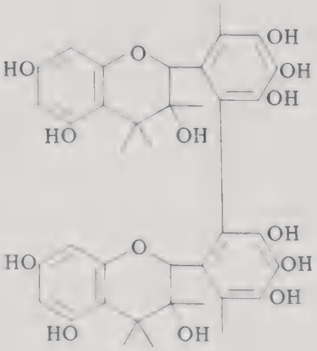
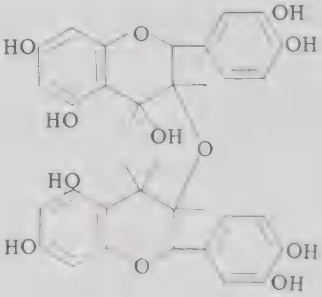


(VII)



(VIII)

same part structure (*21–23*). The mode of linkage in these polymers is not yet known.

Catechins and monomeric leucoanthocyanins can undergo polymerization in several different ways, and this undoubtedly adds to the complexity of the final product. The leucoanthocyanins are characterized by the fact that they produce anthocyanidins on treatment with hot mineral acid (*24*). For compounds of the flavan-3,4-diol structure, this involves oxidative dehydration. Anthocyanidins are produced by such treatment both from monomeric flavan-3,4-diols and from polymers of such compounds (*21*, *25*), but the yield is poor (15–25%) and much of the material is transformed into a dark, brownish red polymer called phlobaphene. A similar polymeric product is also formed by treating catechins with hot mineral acid, and Freudenberg and his collaborators have shown that in this case condensation occurs to give a link between C-2 of one molecule and C-6 (or C-8) of another as shown (V) and furthermore that all flavans which have both a 7- and 4′-hydroxyl group will undergo the reaction (*16*).

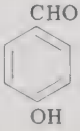
Recent work by Hathway (*22*, *26–28*) has shown that in neutral solution, on the other hand, catechins polymerize by the condensation of quinones formed by autoxidation. The same condensation can be brought out more rapidly enzymatically with phenolase, which Hathway has suggested is the way polymerization is effected *in vivo*. Catechin itself yields quinones which polymerize head to tail, i.e., are linked between the C-2′ of one nucleus and the C-6 of the other (VI), whereas the gallocatechin quinones appear to polymerize tail to tail, by linking of C-2′ with C-6′ (VII). These conclusions were confirmed by experiments with model substances. The suggested structure of the gallocatechin polymer is of interest since Roberts has shown that gallocatechin-3-gallate forms what he considers to be a dimer during the processing of tea (*29*).

Polymeric leucoanthocyanins may be formed by similar processes, but in this case other alternatives must be considered, since, as mentioned above, the polymers themselves still yield monomeric anthocyanidins, albeit in reduced yield, on treatment with hot mineral acid. This indicates that some of the flavan units must be joined by other than C—C links. One possibility is that certain of the units may be attached by ether links to a polymeric core, and Forsyth has shown (*30*) that one of the leucoanthocyanins in the cacao bean is linked to catechin in this way (VIII). This finding indicates that the condensed tannins may, in some cases, be copolymers of catechins and monomeric leucoanthocyanins as suggested above, especially since it has been recently reported that the two monomers generally occur together (*31*). The possibility that gallic or ellagic acid residues are attached by ester links to such polymers should not be ruled out (*15*, *32*).

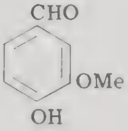
b. Lignin. Lignin occurs in the woody tissue of trees and other plants as a cross-linked, three-dimensional polymer of high molecular weight. Studies on the fine structure of the cell wall have shown that it is associated along with noncellulosic carbohydrates with the cellulose microfibrils of both the primary and secondary cell wall, it being mainly located in the middle lamella (*33*).

Depending on the plant from which it is obtained, it yields on oxidative degradation with alkaline nitrobenzene under pressure one or more of the three aldehydes *p*-hydroxybenzaldehyde (IX), vanillin (X), or syringaldehyde (XI). On reductive degradation, phenylpropane or reduced phenylpropane nuclei with the same hydroxylation and methoxylation patterns are obtained (*34*). From such information it will be seen that lignin contains building stones with the same hydroxylation-methoxylation substitution pattern as that found in the widely occurring cinnamic acids (Table I), and which thus bear the same formal relationship to the B ring of the flavonoids.

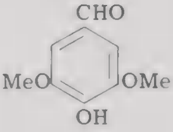
That lignin can be formed from such units is not now in doubt (*35*), but so far it is not possible to deduce a reliable structure for lignin from any one plant. The lignin from conifers yields mainly vanillin (~ 20%) on alkaline nitrobenzene oxidation, but the minor constituents isolated from this reaction includes congeneric substances linked through position C-5 to carbon, such as 5-formylvanillin, dehydrodivanillin (XII), and 5-carboxyvanillin (*36, 37*). The yield of vanillin and other aldehydes from model compounds has been shown to be mainly determined by their mode of linkage in the model compounds used, rather than to any instability of the aldehydes themselves under the conditions of the oxidation (*38*). These findings have led to the concept (*39*) that linkages in lignin are of two types: "open," in which one or more of the carbon atoms of the C_3 side chain of two phenylpropane units are linked together (cf. lignanes, Table V); and "condensed" in which one of the carbon atoms in the aromatic nucleus (usually C-5) is linked either to an equivalent site in the second phenylpropane unit or to a side-chain carbon atom.



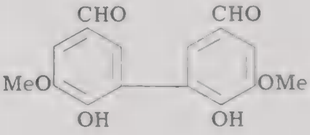
(IX)



(X)



(XI)



(XII)

Freudenberg, in a notable series of researches, has lent great weight to this hypothesis by demonstrating by *in vitro* experiments that coniferyl alcohol (Table V) can couple to yield insoluble dimers of types which might be expected to be the secondary building stores of spruce lignin (*40*). Furthermore, he and other workers, especially

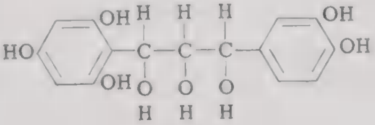
Neish, using radioactive techniques have demonstrated that many such phenylpropane compounds are transformed into lignin *in vivo* (*35*). Nevertheless evidence is accumulating that lignin, and other phenolic polymers, are formed directly in the position where they are found, from sugars (*41–43*).

C. The Biosynthesis of Flavonoid Compounds

1. *Introduction*

Earlier theories on the biosynthesis of flavonoid compounds were based on the fact that numerous instances were known in which two or more flavonoid compounds having common hydroxylation patterns were found in the same plant. Thus Sando (*44*) showed that the 3-galactosides of both quercetin and cyanidin (Table I) were present in the skin of Jonathan apples and concluded that, on ripening, the flavonol was transformed into the anthocyanin.

Although such theories did not throw light on the pathways by which flavonoids were fabricated from elementary building stones, they did underline the probability that only few such pathways existed. Robinson (*45*) suggested that, for anthocyanins, one such pathway was the condensation of two molecules of hexose sugar with one molecule of triose to yield what he termed the "normal hypothetical intermediate"



(XIII)

(XIII) which has the same level of oxidation as the carbohydrate precursors. It also has the phloroglucinol pattern of hydroxylation of the A ring, and the 3,4-dihydroxy pattern of the B ring found in so many flavonoid compounds (Tables I and II). This and other early theories as to the origin of the carbocyclic C_6 fragments have been adequately discussed by Geissman and Hinreiner (*5*), who pointed out the difficulty in propounding a simple theory that would account for the attachment of a C_3 unit both to ring B, with the formation of the 3,4-hydroxylation pattern, and to ring A, leading to the symmetrical trihydroxy structure. They suggested that the precursors of ring A and ring B in flavonoid compounds were probably different, and this proposal has been remarkably substantiated by the advances made in the study of aromatic and flavonoid biosynthesis in recent years (*46*).

2. *The Biosynthesis of the C_6 (B)-C_3 Unit in Flavonoid Compounds*

The structural relationship of the cinnamic acid and other C_6–C_3 compounds to the flavonoid compounds on the one hand, and to the primary building units of lignin on the other, has been pointed out earlier. Consideration of the structures reveals that although it is relatively easy to advance plausible hypotheses for the biosynthesis of both the B ring of flavonoid compounds and lignin from aromatic precursors of the phenylpropane type having suitably oriented hydroxyl groups, no clue appears to be given as to the way in which the aromatic ring itself can be derived from the products of primary metabolism.

In recent years the study of aromatic biosynthesis has received wide attention, and the main features of the pathways by which nonaromatic precursors are converted into compounds containing benzenoid-type rings are now known. In a series of outstanding papers (*47*), Davis and his co-workers have shown that in the coliform bacterium, *Escherichia coli*, the aromatic ring of the amino acids phenylalanine and tyrosine are elaborated from the hydroxy carbocyclic acid shikimic acid (XIV). The pathway by which the latter compound is formed starts from the condensation of a triose and a tetrose and proceeds via 2-keto-3-deoxy-7-phospho-D-glucoheptonic acid as shown (Fig. 1). Subsequent stages in the aromatization are the addition of the C_3 side chain, arising from pyruvic acid, to C-1 of shikimic acid or its biological equivalent (i.e., an activated form), followed by dehydration to yield the isolable prephenic acid (XV). This latter acid was shown to be the direct precursor of phenylpyruvic acid, and hence by transamination phenylalanine, since it decarboxylates quite rapidly under mild acidic conditions and rearranges to give a quantitative yield of the keto acid. It is believed, however, that the route to tyrosine may be somewhat different in that *p*-hydroxyphenyllactate may be involved.

It is now accepted by many workers that shikimic acid (and hence the "shikimic acid pathway") is the precursor of the primary aromatic building stones of many phenolic compounds. Support for this is found in the wide distribution of shikimic acid and related cyclohexane carboxylic acids in plant tissues (*48*, *49*) and the fact that the acid has been shown in isotopic studies to be an actual precursor for the formation of lignin in wheat (*Triticum vulgare*) and sugar cane (*Saccharum officinarum*) (*50*, *51*) and for quercetin in buckwheat (*Fagopyrum esculentum*) (*52*). At the same time a number of radioactive C_6–C_3 compounds, such as phenylalanine, cinnamic acid, and ferulic acid, have been shown to be incorporated into the C_6(B)-C_3 portion of

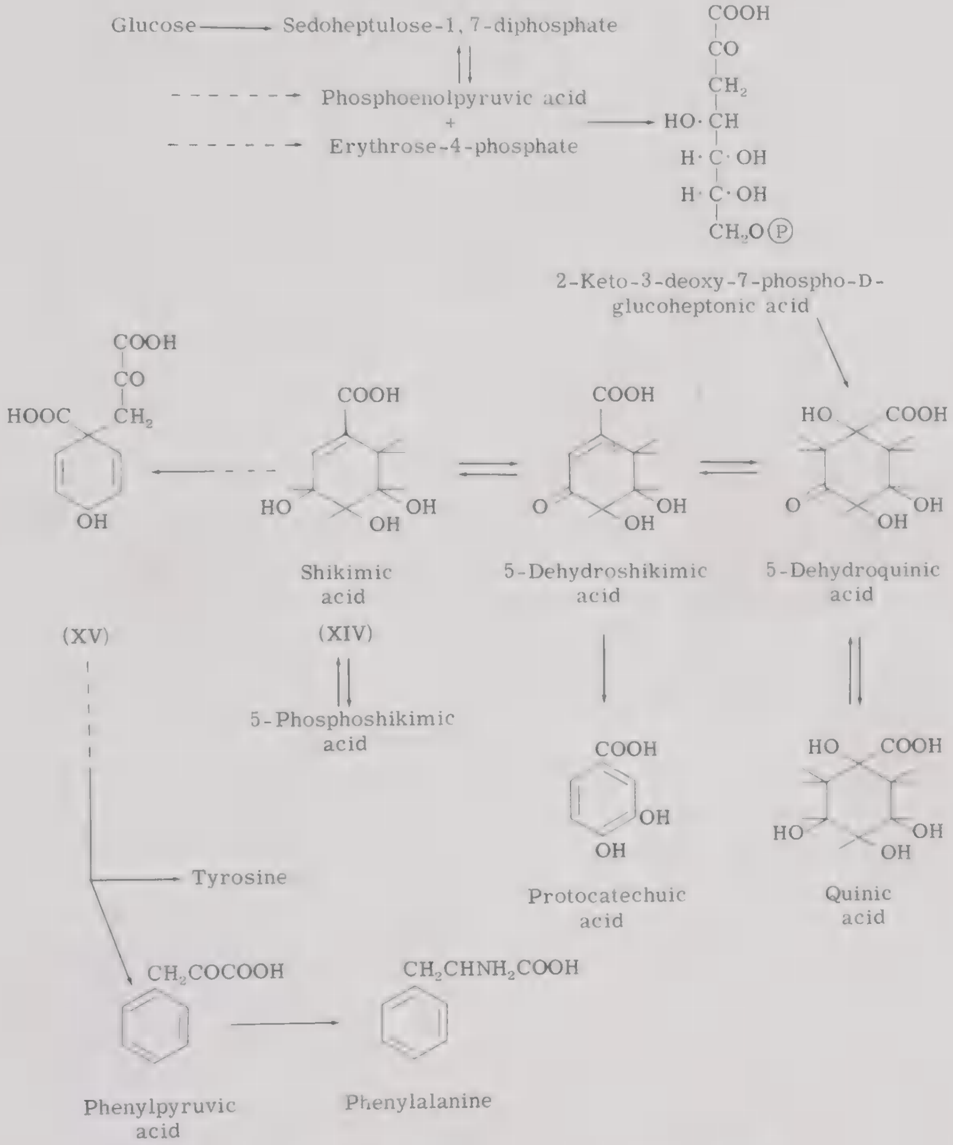


FIG. 1. The route of aromatic biosynthesis.

flavonoid compounds and into lignin, and elaborated into the widely distributed cinnamic acids (*46*) (Table VI).

It may thus be concluded that these compounds, which undoubtedly arise from the shikimic acid pathway, are transformed into the C_6–C_3 portion of flavonoids, to the cinnamic acids and related coumarins, and into the primary building stones of lignin. Before discussing the possible steps by which the final products are elaborated, attention must be

TABLE VI

The Biosynthesis of Flavonoid Compounds from C_6-C_3 Precursors

C^{14} Precursor fed	Radioactive compound isolated and plant fed								
	Quercetin				Cyanidin		Chlorogenic acid	Scopoletin	
	Buckwheat (52)[a]	Buckwheat (66)	Tobacco (66)	*Prunus avium*[b] (77, 78)	Red cabbage (75)	*Rhus glabra* (76)	Tobacco (66) (79)	Tobacco (79)	Wheat[c] (76)
Shikimic acid	0.55[d]								
L-Phenylalanine	1.0	+[e]	+[e]				+	+	
Cinnamic acid	2.5			+	+				
p-Coumaric acid	2.8					+			
o-Glucosyl-*p*-coumaric acid						+			
o-Glucosyl-*p*-coumaryl alcohol						+			
o-Glucosyl-*p*-coumaryl aldehyde						+			
Caffeic acid	19.2		+				+		
Ferulic acid	876								+
Sinapic acid	64								

[a] Numbers in brackets are references

[b] Activity also found in (+) catechin, kaempferol (see Table II), and galangin (see Table III)

[c] Activity also found in tricin (see Table II)

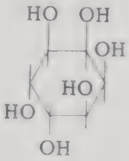
[d] Relative dilution values; L-phenylalanine = 1. The lower the dilution value, the more efficient the precursor

[e] DL-phenylalanine

given to the way the A ring of the flavonoid molecule is synthesized in plants.

3. *The Biosynthesis of the $C_6(A)$ Unit in Flavonoid Compounds*

As a result of the suggestion of Robinson, many workers have postulated that phloroglucinol or a compound of the cyclitol type, i.e., *meso*-inositol (XVI), was the precursor of the A ring in flavonoids (*5*). A series of experiments with quercetin-synthesizing mutants of the green alga *Chlamydomonas eugamatos* appeared to lend support to this theory (*53*), but attempts to confirm this work in other laboratories have not been successful (*54*), and more recently Weygand *et al.* showed that *meso*-inositol-1-C^{14} was not incorporated into either cyanidin in red cabbage (*Brassica rubra*) or catechin in tea (*Camellia sinensis*) (*55*, cf. *56*).



(XVI)

An important contribution to the problem of the biosynthesis of hydroxylated aromatic compounds, including flavonoids, was made by Birch and Donovan (*57*), who suggested that such rings could be considered to be derived from the head-to-tail linkage of acetate units followed by ring closure, as originally postulated by Collie (*58*). Thus addition of three acetate units to a cinnamic acid residue could yield, depending on the mode of cyclization, a flavanone (Table I) or a stilbene (Table V) (Fig. 2).

The resuscitation of the acetate hypothesis followed from the discoveries that acetate was involved in fatty acid (*59*) and cholesterol (*60*) biosynthesis and that the formation of polyacetate units (or their reduction products) could be readily carried out *in vitro* through the activation of acetate by coenzyme A (*61*). As Birch pointed out (*62*), phenolic compounds are more suitable than steroids and fats for studying the incorporation of acetate, since removal of oxygen from an aromatic ring should not occur with such ease as its removal from aliphatic or alicyclic systems. Indeed, using microorganisms, Birch and his co-workers and others have shown that the theory is substantially correct in the case of the synthesis of many hydroxyaromatic and oxygen heterocyclic compounds ranging from 6-methylsalicyclic acid (*63*) (XVII) to griseofulvin (*64*) and alternariol (*65*) (XVIII).

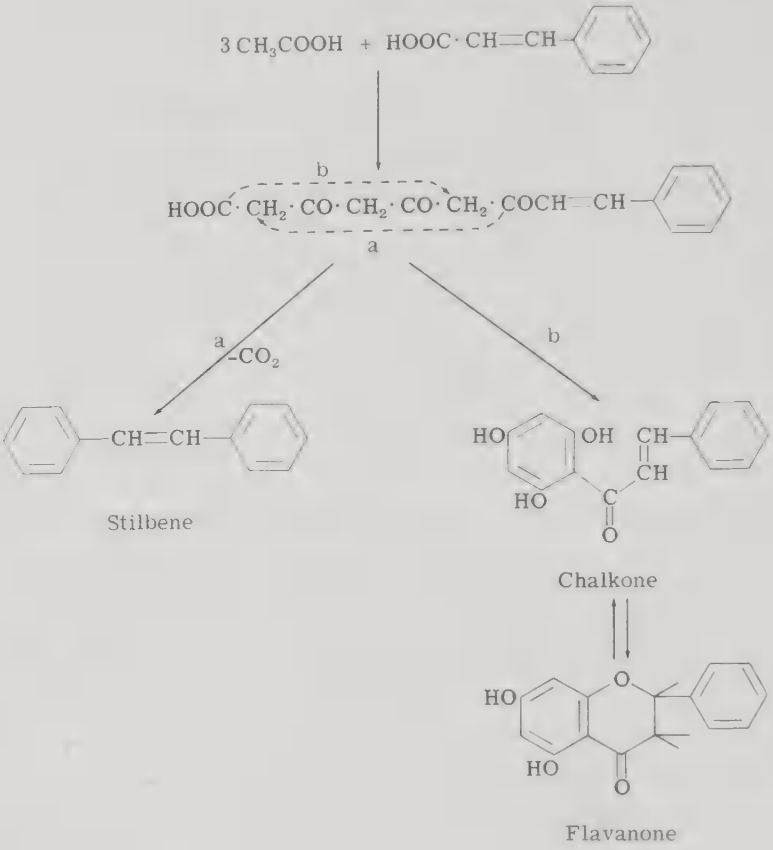
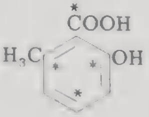
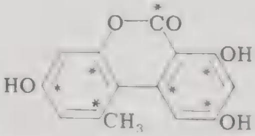


Fig. 2. Biosynthesis of ring A of flavonoids from acetate.

The biosynthesis of ring A in flavonoid compounds from acetate has been studied in several laboratories, and there appears to be no doubt that Birch's original suggestion was a correct one and that the oxygen-bearing carbon atoms of the C_6(A) originate from acetate CO, and the



(XVII)



(XVIII)

other carbon atoms from acetate methyl (e.g., *56*). It should be noted in passing that the C_6–C_3 compounds (e.g., caffeic acid) are not formed by direct condensation of a C_2 unit with shikimic acid (*66*), as suggested earlier (*67*).

It is worth while to consider in more detail the formation of aromatic rings from acetate. Although acetate has been shown to be incorporated not only into aromatic and oxygen-heterocyclic rings, but also into terpenes (*68*), sterols (*60*), carotenoids (*69*), and long-chain fatty acids (*59*), only the pathway to the latter compounds is known with any degree of certainty (*70*). In the case of fatty acids the synthesis follows a route parallel with, and in the opposite direction to, their known methods of breakdown and differs only in so far as an initial carboxylation step from acetyl CoA produces malonyl CoA (Fig. 3). Thus the commonly occurring fatty acids are synthesized in biological tissue by stepwise addition and *complete reduction* of acetate units, although the terminating mechanism, whereby the chain length is regulated to C_{16} or C_{18}, is at present unknown.

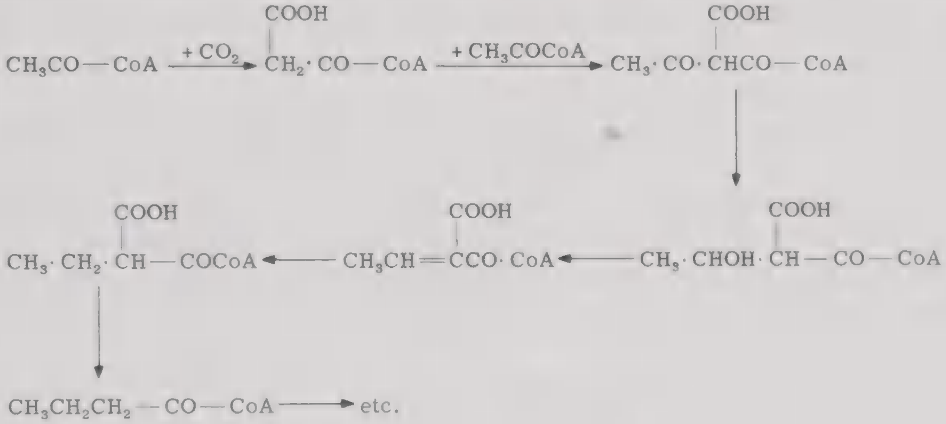
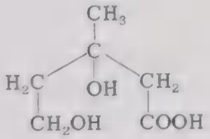

COOH
COOH
$CH_3CO—CoA \xrightarrow{+CO_2} CH_2\cdot CO—CoA \xrightarrow{+CH_3COCoA} CH_3\cdot CO\cdot CHCO—CoA$
COOH
COOH
COOH
$CH_3\cdot CH_2\cdot CH—COCoA \leftarrow CH_3CH{=}CCO\cdot CoA \leftarrow CH_3\cdot CHOH\cdot CH—CO—CoA$
$CH_3CH_2CH_2—CO—CoA \longrightarrow$ etc.

Fig. 3. Biosynthesis of fatty acids.

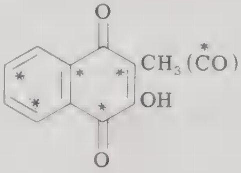
In the case of isoprenoid compounds it has been shown that mevalonic acid (XIX) is an important intermediate in the transformation of acetate into the final product (*60*). The exact mode of synthesis of mevalonate is not known, but it probably arises by reduction of the corresponding dicarboxylic acid (*68*). The route from mevalonate to polyisoprenoid compounds is even more uncertain (*71*, *72*), although isotopic experiments indicate that the terminal carboxyl group is lost during condensation, the β-methyl group being the source of the lateral



(XIX)

methyl substituents in the final product (73). It is plain, however, that here again the oxygen of acetate, or mevalonic acid, is lost during synthesis.

The incorporation of acetate into aromatic rings must proceed by a different pathway from those discussed above, since, as was pointed out earlier, the main tenet of the acetate hypothesis as applied to phenolic substances is the fact that oxygen is not readily removed from aromatic rings (74). Even so, it is possible that the synthesis of aromatic rings and the C_6(A) ring in flavonoid compound might start in the same way as the synthesis of the straight-chain fatty acids. A distinction must be made between the flavonoids in this respect and other acetate-engendered compounds. These other substances are noteworthy in so far as they contain C-methyl groups or larger side chains (often of the isoprenoid type) or may be presumed to arise by loss of a terminal carboxyl group (e.g., the stilbenes, Table V), and in many cases have lost one or more of the carboxyl oxygens of the acetate precursor, e.g., droserone (XX). Furthermore they are built from varying numbers of acetate units [cf. (XVII) and (XVIII)].



(XX)

For these latter substances, polyacetate linkage may indeed be formed by condensation of CoA residues without concomitant reductive steps, but attempts to incorporate polyacetate residues into flavonoids has met with scant success (75).

The biosynthesis of flavonoid compounds may take place in some such manner as that described by Watkin *et al.* (56). That is, the *essential step* is the linking of a C_6–C_3 unit (most probably as a CoA derivative) to the three acetate units required for the formation of the C_6(A) ring. Regardless of the way in which the 2-C units are added, it is certain that the resulting intermediate has a particular orientation that not only determines the formation of the aromatic and heterocyclic rings, but also acts as a regulating mechanism to limit the number of acetate units incorporated.

It is now pertinent to consider in some detail the structure of the C_6–C_3 intermediates involved in flavonoid biosynthesis. Neish and his co-workers showed that L-phenylalanine was more efficient, as judged by dilution values, than cinnamic acid or hydroxycinnamic acids in the formation of quercetin in buckwheat (*52*) (Table VI). It thus appears that the state of oxidation of the C_3 portion of the molecule is of great importance. It was suggested that it was unlikely that phenylalanine itself was a direct precursor of quercetin, but that it formed part of a common metabolic pool of C_6–C_3 compounds from which the precursors of flavonoids and of lignin could be formed (*76–80*). *p*-Hydroxycinnamic acid appeared to be a better precursor than cinnamic acid itself, and since the majority of flavonoid compounds (Table II) contain the 4′-hydroxy group in the B ring it is possible that the common pathway of flavonoid metabolism is dependent on *prior* hydroxylation in this position of the C_6–C_3 precursor.

The cinnamic acids and the building stones of lignin are likewise commonly substituted with a *para* hydroxyl group and it may be assumed that the immediate precursor of these compounds also is a *p*-hydroxyphenylpropane derivative, the state of oxidation on substitution of the C_3 portion being as yet undetermined. Neish and his coworkers (*80–82*) have examined a number of phenylpropane compounds as precursors of lignin in wheat and have shown that among unsubstituted compounds (+)- or (−)-phenyllactic acid is more efficient than any of the other variants tried, being of the same order as L-phenylalanine itself (Table VII). It may be mentioned here that whereas monocotyledons could use both L- and D-isomers of the hydroxy acid, dicotyledons showed a marked preference for the L-form (*82*).

In other experiments on lignin biosynthesis with hydroxylated and methoxylated C_6–C_3 compounds, the Canadian workers observed a variation in the incorporation depending on which unit of lignin was examined. For example, sinapic acid was nearly five times more efficient than phenylalanine as a precursor of syringyl residues in maple, but only one-eighth as good for vanillin (*81*) (Table VII). Similar results were obtained with *p*-hydroxycinnamic acid and ferulic acid, which were efficient precursors of *p*-hydroxybenzaldehyde and vanillin units in wheat lignin and less so for syringyl residues.

These results would seem to indicate that although the plant can utilize a number of variously substituted C_6–C_3 units for synthesis of the different building units in lignin—even those which require the removal of oxygen from the aromatic ring—the highest efficiency is shown with those compounds which have the same substitution patterns as the units themselves, and it follows from this that substitution *precedes* polymerization. It should be noted, however, that, compared with phenylalanine, tyrosine is a poor precursor for lignin except in the case of one member of the Gramineae (*Calamagrostis inexpausa*) (*73*), and for quercetin (*56*), and hence in most cases some modification of the amino acid side chain must take place prior to hydroxylation of the benzene nucleus.

Returning to the flavonoids, little study has been made of the relative *efficiency* of various compounds of the C_6–C_3 type to act as precursors, but it should be assumed that transformations found in lignin biogenesis may well take place also in the case of the simpler molecules. It should be emphasized that the regular pathways of synthesis of flavonoid and related compounds *in vivo* may be difficult to discover because of the diverse ways in which plants can carry out synthetic reactions. Thus, although it has been shown that caffeic acid yields quercetin in buckwheat (52) (Table VI), it should not be generally supposed that under normal conditions this transformation is part of the operative path to the flavonoid in the plant. Again it has been found that the feeding of unlabeled caffeic acid along with radioactive phenylalanine to tobacco leads to greater incorporation of activity in the chlorogenic acid isolated than is the case when the amino acid is fed alone (83). As a third example, the incorporation of tyrosine into lignin was found by Neish *et al.* (*81*) to be between 65 and 375 times less than that of phenylalanine in six species of plants, as judged by comparing the specific activities of the isolated aldehydes; Isherwood (*84*),

TABLE VII

Biosynthesis of Lignin from C_6-C_3 Precursors [a]

Compound fed	Relative dilution values of activity [b] in aldehydes isolated from lignin				
	Wheat			Maple	
	p-Hydroxy benzaldehyde	Vanillin	Syringaldehyde	Vanillin	Syringaldehyde
L-Phenylalanine	1.2	1	0.9	1	2.5
(+)-Phenyllactic acid	2.8	2.8	1.8	—	—
(-)-Phenyllactic acid	1.6	2.1	1.4	1.5	4.0
Cinnamic acid	1.1	2.1	2.0	1.8	9.1
p-Coumaric acid	0.6	1.7	1.4	2.4	6.2
Caffeic acid	15.4	1.1	1.4	2.0	4.9
Ferulic acid	16.4	0.9	2.4	1.1	4.6
Sinapic acid	18.9	426	0.9	8.4	0.5

[a] Adapted from Neish *et al.* (*80–82*).

[b] Activity in vanillin arising from L-phenylalanine = 1.

on the other hand, has shown that tyrosine is a good precursor of lignin in oat coleoptiles whereas phenylalanine is a poor one, incorporation being judged in this case by observing lignin deposition *in situ* on ultraviolet microphotographs of tissue sections. These examples point to the extreme care needed in interpreting experimental results and underline the comments made by Kremers (*43*): "As soon as one tries to think comprehensively about the botanical setting of the lignin problem many more questions arise than have been answered." A similar statement could be made about flavonoids.

4. *The Biosynthesis of Specific Groupings in Flavonoid Compounds*

Many features of the variation in the hydroxylation pattern of flavonoid compounds have been adequately discussed by Bogorod (*46*). Thus *ortho* hydroxylation of existing *para* hydroxyl groups in the B ring in flavonoids would be expected to be mediated by phenolase (*85*). The production of the *vicinal* trihydroxy grouping in this ring is however as yet unexplained since no enzyme system has been discovered which catalyzes the oxidation of aromatic rings containing *ortho* dihydroxy groups to yield such compounds. The insertion of adventitious hydroxy groups in the A ring (Table IV) probably takes place before the closure of the pyran ring under the influence of a laccase-like (parahydroxylase) enzyme. Removal of hydroxyl groups from aromatic rings is undoubtedly difficult (see p. 804), and in those flavonoids that lack hydroxyl groups in the A ring (Table II), such groups are presumably absent from the precursors before they undergo the process of aromatization.

The formation of *O*-methyl ether groups need not concern us here since they are presumably formed by the well-known process of transmethylation from S-adenosylmethionine (*87*, *88*); the formation of other ether groups which are isoprenoid in nature must wait on further research before adequate explanations can be adduced. Methyl groups directly linked to carbon are most probably introduced from C_1 donors like methionine, choline, or formate (*86*, *88*).

The formation of glycosides probably takes place by transglycosidation reactions mediated by uridine triphosphate. Several instances have been reported of the glycosidation of phenolic compounds which were introduced *in vivo*, and it appears that the mechanism is in part relatively unspecific in that sugar can be added to a variety of "foreign" phenolic compounds (*89*, *90*). Genetical evidence (*91*) shows however that glycosidation of naturally occurring flavonoids takes place in a highly specific manner, but whether such groups are added prior to the elaboration of the flavonoid skeleton, or after is not yet known, and although it seems probable that di- and triglycosides are built up from monoglycosides by successive linking of single sugars, there is as yet no clear evidence that this is so. It seems likely, too, that the precise nature and position of the sugar residue is physiologically important and may, in fact, have a bearing on the nature of the flavonoid aglycon that is in due course elaborated.

5. *The Biochemical Significance of Specific Groupings*

Every chemical feature by which one flavonoid molecule differs from another implies one step more or one step less in the biosynthesis. Genetical work on *Neurospora* has shown that there is often more than one intermediate involved in transformations of this type (cf. 92). It is noteworthy that the commonest flavonoids are, as a rule, the more highly oxidized representatives of their particular series—flavonols of the series beginning with dihydrochalkones, leucoanthocyanins of the flavanols. Everything points to the formation of less oxidized representatives being due to *deficiency* in one or more oxidative steps, rather than to one or more steps of reduction being *gained*. It is permissible, in fact, to envisage the evolution of the vascular plants *from* "primitive" ancestral forms (still, in all probability, represented among existing flora) carrying the complete apparatus necessary for the elaboration of the flavonols and leucoanthocyanins, *to* those having few of any flavonoid constituents other than the reduced flavone and flavanone series.

Unit functions in flavonoid compounds which appear to be of special importance in taxonomy are the coumarin, the methylenedioxy, and the furanoxy rings; the additional hydroxyl groups in positions 6 or 8 of the A ring of flavonoids; the absence of the 5-hydroxyl groups; and the absence of the 4′ hydroxyl group from the B ring. All examples of these are restricted to a small number of taxa, and this might indicate a possible relationship between such taxa.

According to the present trend of thought, the presence of each such unit function would be determined by the activity of a single enzyme system and that, in its turn, by the action of a single gene. It is, in fact, significant that so many unit functions in flavonoid compounds have been shown to depend on the presence of one particular gene in the dominant state (see below). As survey data accumulate, and as the work on the chemical genetics of flower colors and other subjects grows, it should be possible to identify with necessary certainty what are the unit functions and to isolate the unit enzyme systems that determine the presence of these functions. One such unit enzyme, for instance, which can be postulated with confidence is that responsible for the production of *vicinal* trihydroxy groups mentioned earlier. This enzyme has so far not been looked for by the biochemist, but it certainly must exist in large numbers of vascular plants and equally certainly must be absent from a great many more. Another unit enzyme which can be postulated is that responsible for the C—C linkage in the biflavonyls (Table IV). The isolation of such enzymes as these would be of the utmost interest in comparative biochemistry since the unit functions they control are so selectively and significantly distributed in nature.

II. The Distribution of Flavonoid Compounds

A. IN HIGHER PLANTS

1. *In One Plant*

Flavonoid compounds have been isolated from every part of plants, but it is obvious when an individual subject is observed that there is a difference in flavonoid pattern in the various anatomical tissues. This is most obvious when the plant has deeply colored flowers pigmented with anthocyanins, since although these compounds are frequently present in the epidermis of the stem and in the leaf veins or in the immature leaf, they are usually absent from the greater part of the plant.

Erdtman (*93*) in recognizing this difference has suggested that a prerequisite of chemical taxonomy is that the reference compounds should be produced from all samples of the species in question, grown under comparable conditions. He recommends that the chemist should pay attention to those natural products which occur in the phylogenetically old, comparatively unspecialized organs, such as the "dead" parts of plants, for example, heartwood or bark, which are largely independent of seasonal variation or other external influences. Against this must be set the fact that not all types of plants have tissues which are woody or which can be considered physiologically old or unspecialized, and furthermore it is possible that heartwood itself is not independent of outside influence, since Chattaway (*94*) has suggested that it is formed as a result of fungal attack. Hasegawa has also given evidence that certain flavonoid constituents may vary both qualitatively and quantitatively in the wood of different trees of the same species (*Prunus yedoensis*) (*95*). Bate-Smith (*11*), has pointed out that although there is a marked variation between one tissue and another, it is reasonable to assume that the chemical constituents of the leaves will be more fairly representative of the plant as a whole, and therefore more suitable for consideration in questions relating to comparative physiology and taxonomy, than those in storage tissue or reproductive organs. He pointed out that questions of homology do of course arise, and leaflike organs (e.g., phyllodes of *Acacia* spp.) have to be treated as leaves for the purposes of survey. In support of this view it was observed that in *Dahlia variabilis,* the leaves contained the same flavonoid compounds regardless of the color or flavonoid content of the flower (*96*).

It is not necessary here to devote much time to enumerating the instances in which workers have isolated one flavonoid from one tissue, and a second from another part of the plant. The more interesting cases of this phenomenon have been enumerated by Geissman and Hinreiner (*5*). A notable contribution to the distribution of flavonoid compounds has been given by Griffiths in *Theobroma cacao* (*97*), and his results are shown in Table VIII. These results illustrate, on an unusually broad front, the differences which might be expected in woody plants which contain flavonols, cinnamic acids, anthocyanidins, catechins, and leucoanthocyanins.

Williams (*98*) has summarized his analyses of the various tissues of the members of the subfamily Poimoideae of the Rosaceae (*99*) by considering the distribution of the three classes of common flavonoids, the flavonols (F), the hydroxycinnamic acids (C), and the catechins and leucoanthocyanins (L): leaves contain $F = C > L$; bark $L \gg F > C$; wood is like bark but has less total phenols; fruit $C > L > F$.

All these observations taken together indicate that the cells of the plant do not necessarily contain the same flavonoid constituents, and it would appear therefore that the possibility must be considered that each cell fabricates its own pattern of phenolic substances from relatively simple common precursors present within it or translocated to it from other organs in the plant (*32*). The other possibility, that phenolic compounds themselves are translocated, must not be overlooked (*22*), but Williams has reported (*100*) that in graft hybrids of pear and quince, the specific characteristic flavonoid compounds of neither stock nor scion were translocated across the graft. True sexual hybrids of apple and pear, on the other hand, had the specific flavonoids characteristic of both species (*101*). The amounts of each compound present were dependent on the nature of the cross; thus in the triploid hybrid (diploid pear × tetraploid apple) the flavonoids characteristic of the pear were relatively weaker than in those hybrids in which both parents were diploid or tetraploid. This situation should be contrasted with the dominant recessive relationship shown in intraspecific crosses described in the next section.

The variation in plants makes it difficult for over-all generalizations to be made regarding the distribution of flavonoids in the various tissues.

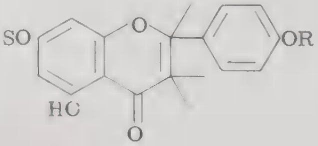
TABLE VIII

Distribution of Flavonoid and Related Compounds in the Tissues of *Theobroma cacao*

Compound	Tissue										
	Mature pod, outer wall	Mature pod, inner wall	Cotyledon	Flower	Young leaf	Old leaf	Green stem	Bark	Sapwood	Heartwood	Root
In methanolic extracts											
Leucoanthocyanin L_1	+ +	+	+ +	+ +	+ +	+	+ +	+	+ +	+	+
Leucoanthocyanin L_2	+ +	+	+ +	+ +	+ +	+	+ +	+	+ +	+	+
3-β-D-Galactosidylcyanidin			+ + + +	+ +	+						
3-α-L-Arabinosidylcyanidin	+		+ + + +	+ + +							
Rutin				+ +	+ +						
Epicatechin	+ +	+	+ + + +	+ + +	+ + +	+	+ +	+	+	+	+
p-Coumarylquinic acid			+		+ + +	+					
Chlorogenic acid					+ + +	+	+		+		
Neochlorogenic acid						+ + +					
In hydrolyzates											
Cyanidin	+ +	+ +	+ + + +	+ + +	+ + + +	+ +	+ + +	+ +	+ +	+	+
Kaempferol					+						
Quercetin	+	+	+	+ + +	+ +	+			+		
p-Coumaric acid		+	+	+ +	+	+ + +	+ +				
Caffeic acid	+	+ +	+ +		+ + +	+ + +	+ +				+ + +
Ferulic acid	+ +					+ +					
Sinapic acid						+ +					
Gentisic acid	+ +	+	+	+	+ +	+ + +	+	+ +	+ +	+ +	+

and, as stressed above, care must be taken when comparing the flavonoid content of, say, the roots of one species with another. In this case gross morphological changes affected by habit, maturity, climatic and edaphic factors must be considered.

One important cause of variation, even in storage organs, is of course seasonal. Thus it is easy to see that many leaves, especially of certain plant families (e.g., Rosaceae), are noticeably red when immature or change during the autumn. Little quantitative information is available on the seasonal changes in individual flavonoid compounds although Hattori and Shimokoriyama studied such changes in the flower, fruit, and leaf content of flavanone glycosides of *Poncirus trifoliata* (*102*). They found that the content of poncirin (XXI) and naringin (XXII) decreased in the flower during the course of the season, whereas in the fruit the concentration of poncirin reached a peak in mid-June when the fruit was still immature and then decreased until August, only to rise again at full ripeness; very little naringin was found to be present. The most interesting finding was that in the leaves there was twice as much poncirin as naringin at the bud stage, but when the leaves reached normal size at the end of one month they contained equal quantities of poncirin, and instead of naringin, the corresponding flavone rhoifolin (XXIII). As the season developed only rhoifolin remained.

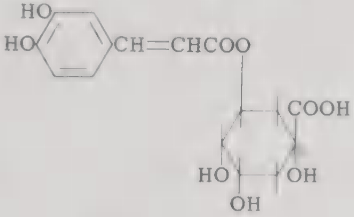


(XXI) R = Me
(XXII) R = H
(XXIII) Flavone corresponding with XXII (cf. Table II)
S = rutinosyl

Hillis and Swain (*103*) have investigated the change in total phenols, leucoanthocyanins, and catechins (flavans) in the leaves of the Victoria plum tree (*Prunus domestica*). Here it was found that the methanol-soluble phenolic compounds increased as the season progressed, the changes following a normal growth curve, with the leucoanthocyanins showing the most rapid rate of increase.

The authors found that a most significant difference existed between leaves on the sunny and those on the shady sides of the same tree, and they adduced evidence to indicate that light intensity plays a part in phenolic, especially leucoanthocyanin, synthesis. They also showed that as the season progressed an increasing part of the leucoanthocyanins became polymerized, finally becoming insoluble in aqueous and alcoholic solvents.

Such findings emphasize the difficulties in obtaining meaningful quantitative data on flavonoid compounds, and this is exemplified by experiments carried out on potatoes (*104*). It was found that the leaves of potato plants grown under glass contained 30–50% less phenols per leaf (*105*) than those grown out of doors, but the content of chlorogenic acid (XXIV) in the tubers was roughly the same in both cases.



(XXIV)

Again experiments on tubers of a single plant grown in the field showed that their chlorogenic acid content varied ±30% whereas that of tubers, even from different plants, grown in culture-solution experiments showed only ±10% variation.

Other environmental conditions undoubtedly affect the over-all production of phenolic compounds (*46*, *106*), in some cases resulting in gross qualitative changes. Attention should be paid also to changes resulting from varying physiological (*107*) or pathological causes (*108*), which may in some cases give rise to the production of flavonoid compounds which would normally be present in quantities below the level of detection.

2. *In One Species*

A large number of papers have appeared concerning the variation of flavonoid compounds in one species. Indeed, Mendel himself made great use of flower color to follow fundamental paths of gene action, and much of the success of plant breeding depends on a knowledge of the genetic factors that control flower color. Largely as a result of the brilliant pioneer researches begun by Wheldale (*109*) and continued by workers at the John Innes Horticultural Institution (*110*), many demonstrations of specific genetic factors that influence the control of synthesis of individual flavonoid compounds have been made. It should be stressed that in work of this kind, the structure of the pigments concerned and the manner of inheritance of the factors for flower color in the particular species must first be established before conclusions as to the action of the controlling genes can be determined.

It is not possible in the space provided to mention every completed piece of experimental work in which these criteria are satisfied, and only two cases will be given here; further examples have been detailed by Paech (*111*).

The variation in the flower color of the diploid *Antirrhinum majus* has attracted the attention of the chemical geneticist for a long time (*112*), and only in recent years with modern techniques has the problem come near to solution with the almost simultaneous publication of work done both in the United States (*113*) and in England (*114*) on the genetic control of the aglycons present in the various color varieties. Small discrepancies in the results of these two workers have been resolved by Sherrat (*115*), whose findings for homozygous plants are summarized in Table IX. Four main color genes were found, denoted *N* (null or *A*, albino), *P* (pink), *M* (modifier), and *Y* (yellow) by the American workers. All genotypes other than the bottom recessive *nn* contained the aurone aureusidin and the flavone apigenin (Table II). The gene *P* is necessary for the formation of both anthocyanin and

TABLE IX

The Flavonoid Aglycons in Genotypes of *Antirrhinum majus* [a]

Genotype [b]	Color of flower	Flavonoid pigment						
		Aureusidin [c]	Cyanidin	Pelargonidin	Quercetin	Luteolin	Kaempferol	Apigenin
PP MM YY	Magenta	+	+		+	+		+
PP mm YY	Pink	+		+			+	+
PP mm yy	Yellow-orange	+ +		+			+	+
pp MM YY	Ivory	+				+		+
pp MM yy	Yellow	+ +				+		+
pp mm YY	Ivory	+						+
pp mm yy	Yellow	+ +						+
nn pp mm yy	Dead white [d]							

[a] Adapted from Sherrat (*115*).
[b] All except last are *NN* types.
[c] +, Present in lip only; + +, present throughout the flower.
[d] Contains *p*-coumaric and caffeic acids.

flavonol. When present with *M*, cyanidin and quercetin are formed; and with *m*, pelargonidin and kaempferol. *M* thus modifies the hydroxylation pattern of the B ring of the flavonoids (other than the aurone) whereas *P* can be thought of as introducing a hydroxyl group at C-3 of the flavonoid skeleton. In the *pp mm* plants only the 4′-monohydroxylated flavone is formed whereas with *M*, luteolin was also found to be present, and this latter compound was also found in *PP MM* varieties. The gene *Y* modifies the amount of aureusidin formed, the quantities being greater in flowers from *yy* plants. The albino *nn* was found to contain *p*-coumaric and caffeic acids, but these are present also in all other genotypes, being in higher concentrations in *mm* plants.

From such data Jorgensen and Geissman (*116*) suggested the scheme shown in Fig. 4 for the biosynthesis of flavonoid compounds in *Antirrhinum majus*. Sherrat (*115*) proposed that "precursors 1 and 2" in Fig. 4 are the A ring fragments of flavonoid compounds. Extra genes may be involved since some English strains showed the presence of quercetin and luteolin in the absence of anthocyanin (*115*), but these have yet to be worked out. It is not necessary to comment on the scheme shown in Fig. 4, except to point out the difficulty of reconciling parts of it with the known facts on the biosynthesis of flavonoids discussed earlier.

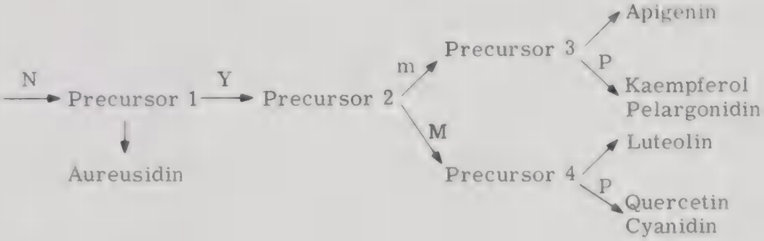


FIG. 4. Action of genes in *Antirrhinum majus*. Scheme adapted from Jorgensen and Geissman (*116*).

Feenstra (*117*) has recently published his work on the flavonoid compounds responsible for the different seedcoat colors in genotypes of French beans (*Phaseolus vulgaris* L.), which have been under investigation for several decades without yielding clear-cut results. The situation he found is more complex than that in *Antirrhinum* in that the genetic factors interact to yield a wide variation in the pigment produced (*118*). Here only the data presented in his original paper will be given. In this the effect of six of the eight possible homozygous genotypic combinations of the factor pairs *C*–c^u, *Sh*–*sh*, and *V*–*v* are shown (Table X): all of them contained a basic ground factor *P*.

The gene *Sh* (shine) gives the beans a creamish color and a glossy appearance, but when exposed to air and light the beans become reddish brown, and it was found that this gene controls the formation of leucocyanidin and leucopelargonidin. The factor *C* is one allele of the multiple allelic series at one locus and controls the production of two kaempferol glucosides, the 3-glycoside and the 3-glucosidoxyloside. The third gene *V* (violet) gave no pigments when present alone, but along with *Sh* gave leucodelphinidin as well as the other two leucoanthocyanins which *Sh* alone produces. The seed coats of the genotype *sh V C* showed the presence of monoglucosides of the anthocyanins delphinidin, petunidin, and malvidin, along with myricetin-3-glucoside and traces of quercetin glycosides. Interactions of *Sh* with *C* or other alleles of the C locus were found to be rather complicated. Similar results have been obtained with *Impatiens balsamina* (*119*).

TABLE X

Flavonoid Compound in the Seedcoats of Genotypes of *Phaseolus vulgaris* [a]

Genotype	Color of seedcoat	Flavonoid compound				
		Leucocyanidin and leucopelargonidin	Leucodelphinidin	Kaempferol, 3-glucoside and 3-glucosidoxyloside	Delphinidin, petunidin, and malvidin 3-glucosides	Myricetin, 3-glucoside and others
Sh Sh c^uc^u *vv*	Cream [b]	+				
Sh Sh c^uc^u *VV*	Cream [b]	+	+			
sh sh CC vv	Lemon yellow			+		
sh sh CC VV	Blue				+	+
sh sh c^uc^u *vv*	White					
sh sh c^uc^u *VV*	White					

[a] Data from Feenstra (*118*).

[b] Have brown ring round hilum, and whole seedcoat turns brown slowly on exposure to air.

3. *In One Genus*

Many recent examples are available of comparisons between the flower or leaf flavonoid pigments of various cultivated and wild species belonging to one genus. It should be remembered that the genetic variability of wild-type plants is usually much less than that found in cultivated plants, and where *consistent* differences are noted in chemical constitution between two closely related types, it is probable that these are distinct species. Nevertheless some difficulty might be experienced in comparing the constituents of one species with those of another where the two species normally had widely differing habitat or were subjected to differing photoperiodic effects. Thus in the genus *Solanum*, to which the potato and tomato belong, some species are tropical, some temperate, and if comparisons were made on material collected in the field, the tropical species might provide a greater content of flavonoids merely as a result of the different conditions of growth. On the other hand, if comparisons are made with material growing in the same botanic garden in a temperate climate, the exotic species grown under glass may show much reduced content of phenolic compounds compared with those from a natural habitat.

Often, however, most of the species of a single genus grow within a limited climatic and geographical area under comparable edaphic conditions, and here valid comparisons can be made. Roberts and his co-workers (*120*) have investigated the flavonoid constituents of species of *Camellia* grown in Assam. In the subsection *Thea*, the most important compounds taxonomically were the triglycosides of the flavonols quercetin and kaempferol, an unknown substance IC which is a gallic acid derivative readily recognizable on a two-dimensional paper chromatogram, and to a less extent depsides of quinic acid with gallic acid, caffeic acid, and *p*-coumaric acid. The flavonol triglycosides were present in relatively large amounts only in the leaves of the China variety of *C. sinensis*, specimens of the Assam variety contained little or none of these compounds, and they were completely absent from a southern variety of tea. This latter variety was the only one referable to the species *C. sinensis*, which contained the substance IC, which was also present in the two other species of the subsection *Thea* (Table XI).

In the other sections examined, *Theopsis* and *Camelliopsis* showed no resemblance to *Thea* except for the presence of gallic and ellagic acids, which are in any case widely distributed in nature. The section *Camellia* also showed no resemblance to tea except for the presence of (—)-epicatechin in two species. The section *Paracamellia* also was quite different except for the presence of the common depsides in *C. kissi* and *C. sasangua*. The close similarity between *C. saluensis* and the latter species does not support their being placed in different sections of the genus. The authors were unable to detect any difference between

C. reticulata and *C. pitardii*, which are morphologically similar. In the section *Thea*, they suggested that *C. taliensis* and *C. sinensis* may not be distinct species, but that *C. irrawadiensis* is certainly so.

Hasegawa (95) has investigated the flavonoids present in the wood of various Japanese species of the genus *Prunus*. As mentioned earlier, he found some quantitative variation in flavonoid content in the different specimens of the same species and also showed that a variation existed between hardwood and sapwood in respect to the ratio of glycosides and aglycons. The *Prunus* species he examined all contained naringenin and aromodendrin and their glycosides, but otherwise there was a variation which coincided, with few exceptions, with the taxonomical sections of the genus. Referring to flavanones *Fa*, flavones *Fo*, catechins *C*, and leucoanthocyanins *L*, he summarized his findings as follows: subsection *Cerasus, Fa-Fo-C-l; Amygdalus, fa-Fo-C-L; Prunophora, Fa-Fo-C-L; Padus, fa-fo-C-l; Laurocerasus, fa-fo-c-l;* the use of capitals or lower case letters indicates the amount of the flavonoids present.

TABLE XI

Distribution of Flavonoid and Related Compounds in *Camellia* Species and Varieties[a]

Species and variety	Flavonoid compound			
	Depsides	IC	Triglycosides	28/18[b]
C. sinensis				
China variety	+	-	+ + +	-
Assam variety	+ +	-	-	-
Southern form	+ + +	+ + +	-	-
C. taliensis	+ + +	+ + +	-	-
C. irrawadiensis	+ +	+	-	+ + +

[a] Data from Roberts *et al.* (*120*).

[b] Probably a gallic acid derivative; code number indicates R_f values in butanol-acetic acid-water and 2% acetic acid.

An interesting correlation between the commonly occurring flavonoid compounds and the species or sections of a genus has been given by Bate-Smith (2) for the genus *Iris* (Table XII). The results show that all the sections examined contained methoxycinnamic acids. The similarity between *Evansia* and *Pogoniris* should be noted, as these sections are regarded as equivalent eastern and western groups. The co-occurrence of leucoanthocyanins with flavonols was found to be restricted to certain species of the section *Apogon*. One species, *I. flavissima* Palt. was especially interesting since it had been placed originally in the section *Pogoniris* and this had been questioned by later workers who, on the basis of cytological studies, suggested it should be in the *Regelia* section; this suggestion is supported by the fact that its flavonoid constituents exactly match the latter section. The over-all biochemical variation within the genus was found to be considerable, but no more than might have been expected with such a spread of variation in root morphology.

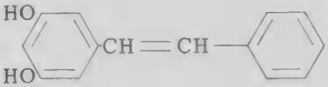
TABLE XII

Distribution of Flavonoid and Related Compounds in Sections of *Iris*[a]

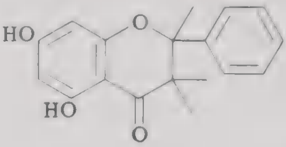
Sections or subsections	Flavonoid compounds				
	Leucoantho-cyanins	Flavonols	Caffeic acid	Methoxy cinnamic acids	Isoflavones[b]
Reticulata	-	-	-	?	-
juno	-	-	-	+	-
Pardanthopsis	-	-	-	+	-
Apogon					
sibirica	+	+	+ -	+	-
spuria	+	-	(+) -	+	-
californica	+	+	+	+	-
hexagona	-	-	+	+	-
laevigata	+	-	-	-	-
foetidissima	-	-	(+)	+	-
unguicularis	-	-	(+)	+	-
ensata	-	-	-	+	-
Regelia	+	-	+	+	-
Evansia	-	-	-	+	+
Pogoniris	-	-	-	+	+

[a] Data from Bate-Smith (*2*).
[b] In rhizomes.

By far the most complete chemical taxonomic study of one genus is that of *Pinus* described by Erdtman and his colleagues. This work has been reported fully elsewhere (*93, 121*), but some brief mention must be made of the interesting findings. All in all, some twelve flavonoid compounds were isolated and their distribution in over half of the species of the hundred or more in the genus have been investigated. The variations in pattern indicated that the subdivision of the genus already recognized by botanists coincided remarkably with the results of chemical classification. For example in the two main subgenera *Haploxylon* and *Diploxylon* the stilbenes pinosylvin (XXV) and its



(XXV)



(XXVI)

monomethyl ether, and the flavanone pinocembrin (XXVI) and the corresponding flavonol pinobanksin were found in all the species examined. However, with two exceptions, only these four compounds were found in the species grouped under *Diploxylon*, whereas in the *Haploxylon* species a much greater variety of flavonoids was found, including not only the dihydrostilbenes but flavones, and noticeably in one subsection C–Me flavones and flavanones.

4. *In One Family*

Erdtman (*93, 121*) has covered a number of other genera of Pinaceae and shown that, where evidence is available, the chemical results are in good agreement with the botanical classification. In the case of many other families of Coniferae, however, the flavonoids do not seem to be as useful taxonomically as other types of compound, especially the terpenes, and although these studies fall outside our scope the papers are essential reading for those interested in chemical taxonomy.

Other surveys on the distribution of flavonoid compounds in families have not been carried out with the same degree of thoroughness as those of Erdtman and his group, but by application of paper-chromatographic and other modern methods, many useful contributions have been made to the study of chemical taxonomy of such groups (e.g., *122–125*).

In many cases the various division of the family can be distinguished chemically by the fact that one or more possesses some flavonoid constituent absent from the other divisions. Hegnauer (*125*) has pointed out that such compounds are of particular value as systematic guides. Of course little use could be made of compounds of unique occurrence, but it appears to us unlikely that such compounds exist; it is more probable that they have merely not been looked for in related species. It would be as unusual for a particular constituent to be formed in one plant and not its close neighbor as it is to find orders with only a single species. Such orders do exist of course (Ginkgoales), but even

TABLE XIII

Distribution of Flavonoid and Related Compounds in Tribes of Rosaceae

Compound	Spiraeoideae		Pomoideae	Rosoideae							Prunoideae	Chrysobalanoideae	
					Potentilleae								
	Spiraeeae	Quillajeae (Exochorda)	Pomarieae	Kerrieae	Rubinae	Poten-tillinae	Dryadinae	Ulmarieae	Sanguisorbeae	Roseae	Pruneae	Chryso-balaninae	Hirtellinae (Parinarium)
Leucoanthocyanins	+ +	+	+ +	+ -	+ -	+	-	(+)	+ +	-	-	LCy - LD +	LCy + LD -
Flavonols	+ +	+ +	+ +	(+)	+ +	+ +	-	+ +	+ +	+ +	+ +	Q + M + +	Q + M -
Caffeic acid	+	- ?	+ +	-	+	+	+	+	+	+	+ +	+ -	-
Ellagic acid	-	-	-	-	+	+	+	+	+	+	-	-	-
Ferulic acid	+ -	-	-	-		+	-	-		+ -	(+) -	-	-

here the phenolic substances examined do not appear to be unique or even unusual in the sense of being absolutely restricted to very closely related orders.

It seems instructive to us to illustrate the theme of chemical taxonomy as applied to families with results on the distribution of the common flavonoid compounds (Table I) in the Rosaceae (*2, 126*). The patterns of the chemical compounds were found to agree with the subdivision of family based on exomorphic characters (Table XIII). This family is distinctive for the rarity of occurrence of trihydroxy-substituted constituents, and those which do occur are confined to very distinct tribes. Other tribes, and some genera, have their own distinctive and, in some cases unique, constituents.

The over-all phenolic pattern is that consistent with the generally woody habit of the family, many of the members being trees or shrubs, somes canes or briars, some herbaceous.

The leaves, especially of the woody species, are rich in flavonols (kaempferol and quercetin only) and leucocyanidin; the only exception in the 200 species examined were *Chrysobalanus pellocarpus* Mey, *Licania rigida* Bent., and *Potentilla anserina* L., which contain myricetin and leucodelphinidin in addition. The herbs and briars of the tribe Rosoideae often contain no leucoanthocyanins, and less often no flavonols or caffeic acid; but this tribe is the only one in the family in which ellagic acid is found, and it is present in all sections except Kerrieae.

Among the specific constituents of the Rosaceae may be mentioned the glucosides of salicylaldehyde and methyl salicylate in the Spiraeoideae; the dihydrochalkones phloretin and hydroxyphloretin (*127*) (see Table IV) in *Malus* and arbutin (hydroquinone glucoside) in *Pyrus* of the Pomoideae. Incidentally, hydroxyphloretin has been isolated only from the subsection Sieboldianae (Rehd). of *Malus*. The Rosoideae contain a glucoside of eugenol (gein), and the Prunoideae an isoflavone prunetin (Table II) and the cyanogenetic glycoside amygdalin. A further interesting feature is the presence of *o*-coumaric acid in certain *Prunus* spp.

5. *In All Higher Plants*

In this section, as in the last, attention will be paid only to the distribution of the commonly occurring flavonoid constituents (Table I). Certain restricted orders have been dealt with in detail by certain authors, notably Reznik, who studied the distribution of flavonoid pigments and the so-called nitrogenous anthocyanins in the Centrospermae (*128, 129*). Reznik has also made a survey of a number of commonly occurring flavonoid glycosides in the leaves, petals, and fruits of over 100 species (*130*). Another useful survey is that made by Gibbs (*131, 132*) on the distribution of syringyl residues in lignin of dicotyledons.

The widest survey of flavonoid compounds in plants which has been made so far is that of Bate-Smith (*2, 11, 126, 133–141*). He has studied the distribution of the commonly occurring flavonoids shown in Table I (excluding cyanidin) and of ellagic acid (II) (*137*) in over 1000 species of flowering plants and certain selected species of nonflowering plants. The occurrence of these overall in monocotyledons and dicotyledons is shown in Table XIV (*11*). It can be seen that the monocotyledons as a group are characterized by having fewer species containing the flavonoid components than the dicotyledons, and many more species which contain the methoxycinnamic acids, ferulic and sinapic acid. This latter point is significant in so far as the monocotyledons are, in general, herbaceous in character, and these two acids have the same hydroxylation-methoxylation pattern as the building stones of lignin (*105*). It is striking that in dicotyledons also the distribution of these methylated cinnamic acids was found to be in general limited to nonwoody families (*2*).

TABLE XIV

Distribution of the Common Flavonoid and Related Compounds in the Angiospermae

Compound[a]	Per cent of species examined from		
	Monocotyledons	Dicotyledons	Total
Leucocyanidin	27	37	35
Leucodelphinidin	8.5	19	19
Kaempferol	26	52	48
Quercetin	28	62	56
Myricetin	3.5	11	10
p-Coumaric acid	55	48	49
Caffeic acid	50	66	63
Ferulic acid	67	33	48
Sinapic acid	57	26	32

[a] See Table I.

The other significant fact which has emerged from this survey is that the presence of leucoanthocyanins in dicotyledons is highly correlated with woodiness (*2, 11, 136*), and even more so with the occurrence of "botanical" tannins (*133*). The over-all distribution in these compounds between woody and nonwoody families (*142*) is shown in Table XV (*11*).

TABLE XV

Distribution of the Various Classes of Flavonoid and Related Compounds in the Angiospermae

Class of compound	Per cent of species examined from			
	Monocotyledons	Dicotyledons		
		Woody	Herbaceous	Total
Leucoanthocyanins	35	61	19	40
Flavonols	41	90	58	72
Caffeic acid	65	93	78	83
Methoxycinnamic acids	51	19.5	46	34
Null	6			2.5

When individual families were considered, it was found that the leucoanthocyanins were absent from the majority of families of the orders Centrospermae, Umbelliflorae, and Contortae and entirely absent from the Rhoeadales, Tubiflorae, Plantagenales, Curcubitales, and Campanulatae. However a large number of woody plants in the last six orders of the Sympetalae (*143*) were found not to contain leucoanthocyanins, and Bate-Smith pointed out that it is clear that consideration of both systematic position and habit determine the distribution of these components. The distribution of flavonols is very similar to that of the leucoanthocyanins, although they are absent from fewer families (Table XVI) (*2*). Also included in Table XVI are a number of less common constituents which are of obvious taxonomic importance.

The over-all picture which arises from this summary is that the commonly occurring flavonoid compounds and closely related cinnamic acids are, as Bate-Smith expressed it (*2*), "a biochemically privileged class." The elaboration of the structure of flavonoids requires as a necessity the operation of two specialized routes to aromatic rings, the unique "triacetate pathway" and the "shikimic acid pathway," the latter being required also for the synthesis of the cinnamic acids and for

TABLE XVI

Distribution of Flavonoid and Other Compounds of Taxonomic Importance in the Orders of Dicotyledons

Engler-Prantl orders[a]	Archichlamydeae												Sympetalae				
	1-16	Centrospermae Cactaceae 17 & 28	Ranunculaceae Rhoeadales 18 & 19	Sarraceniaceae 20	Rosaceae 21	Geraniaceae 23	Sapindaceae 24	Rhamnaceae 25	Malvaceae 26	Parietales 27	Myrtiflorae 29	Umbelliferae 30	Ericaceae 1	Primulaceae Plumbaginaceae 2 & 3	Ebanaceae 4	Contortae 5	6-10
Leucoanthocyanins	+	-	(+) -	+	+	+ -	+ (-)	+	+ -	+ -	+	-	+	+	+ -	-	(+) -
Flavonols	+	+ (-)	+	+	+ (-)	+	+	+	+	+ (-)	+	+	+	+	+	+	+ -
Hydroxy acids	+ (-)	-	+ -	+	+ (-)	+ (-)	+	+ (-)	+	+ (-)	+	+	+	-	+	+	+
Methoxy acids	(+) -	+ +	+		(+) -	(+)	(+)	-	-	(+) -	(+) -	+	-	-	(+) -	+	+ +
Ellagic acid	+	-	(+) -	+ -	+	+	+	+	-	+ -	+	(+) -	+	-	-	-	-
Nitrogenous anthocyanins	-	+ +	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Anthraquinones	+ [b]	-	-	-	+ ? [c]	+ [d]		+ +			-	-	-	?	-	-	+ [e]
Berberine alkaloids	-	-	+ +	-	-	+ [d]	-		-	-	-	-	-	-	-	-	-
Isoflavones	-	-	-	-	+	-	-	-	-		+	-	+		-	-	-

[a] References 99 and 143. [b] Polygonaceae. [c] Leguminosae. [d] Rutaceae. [e] Rubiaceae; Verbenaceae.

lignin. It would appear that some relationship exists between the formation of lignin on the one hand and that of the most common flavonoids, the leucoanthocyanins and flavonols, on the other, since these are found so closely linked in woody plants. If it is assumed that the pathway by which any individual skeleton is formed is the same in all plants in which it is found, then this must be related to a single piece of information in the genetic code of the primeval nucleus. It is possible then that phenolic synthesis was originally determined by a multiple allelic system on the primary chromosomes, in which the allele(s) for methoxycinnamic acid synthesis was dominant to that of lignin, and that the loci of the recessive form of the allele and that of the genes controlling leucoanthocyanin and flavonol synthesis are near enough to ensure that separation by crossing over is a rare event. Obviously many other phylogenetic theories may be elaborated, but before proceeding further we need to know more about the individual steps in the biosynthetic process and about the function of the end products in regard to their advantage to the plant.

B. In Other Classes

With the sole exception of *Chlamydomonas eugametos* mentioned earlier (*53*, *144*), flavonoid compounds in the strict sense have been found only in vascular plants. Fungi and lichens contain a large number of phenolic constituents, especially depsides of phenolic acids, all of which seem likely to be acetate engendered (*145*, *146*). In the algae, gallic acid occurs in *Spirogyra arcta* (*147*) and ellagic acid in *Spirogyra majascula* (*148*) (which may be the same species), but these compounds are not necessarily on the direct route to flavonoid biosynthesis [cf. protocatechuic acid in Fig. 1 and Bassett and Tanenbaum (*149*)]. There is no record of flavonoid compounds in mosses [the "lignin" of mosses is quite different from that of vascular plants (*150*)], but in Pteridophyta flavonols, leucoanthocyanins, and hydroxycinnamic acids occur as freely as they do in the Spermatophyta (*148*). Thus *Equisetum arvense* contains luteolin-5-glucoside, kaempferol-7-diglucoside, and isoquercitrin (*151*), and ferulic acid has been isolated from *E. hiemale* (*152*). Kaempferol derivatives have been found in *Pteridium aquilinim* (*153*), and *Lycopodium clavatum* was shown to contain dehydrocaffeic acid (*154*).

It seems from these examples, that whereas most of the common flavonoid compounds may be expected to occur in all classes of vascular plants, they are virtually absent from the nonvascular ones. This gives further strong indication of the association between the flavonoids and lignin, the characteristic constituent of vascular tissue.

III. Metabolism and Function of Flavonoid Compounds

A. In Plants

Apart from their association with woodiness, the biological role of flavonoid compounds in plants is still for the most part unknown. Various theories have been propounded, but little evidence is at present available as to their correctness. It seems highly probable that the coloring matters in insect-pollinated flowers make these organs more conspicuous to the vectors responsible, especially since many flowers are patterned in such a way as to guide the insect to the anthers and style. However, little seems to be known as to the effectiveness of the coloring matters in attracting insects or as to whether other mechanisms are operative. The function of the coloring matters in edible fruits may also be to make these organs more conspicuous and so aid seed dispersion by animals. The presence of coloring matters in other parts of the plant may be related to their function in the reproductive organs.

Some evidence has been presented for the flavonoid compounds having a function in the sexual processes of the plant. Thus Kuhn and Löw (*155*) have shown that the inability of two varieties of *Forsythia* to cross-pollinate is associated with the presence of rutin in the pollen of one and quercitrin in the other. Further work on these lines is obviously desirable.

Many hypotheses have been put forward relating the flavonoid compounds as substrates for oxidation-reduction processes relating to the function of phenolase. It appears probable, however, that phenolase does not function as a terminal oxidase (*85, 156*) and that if the flavonoid compounds are to be assigned any role it may be in the protective action that their oxidation products impart to damaged tissues (*157, 158*). Thus Byrde and his co-workers have shown (*158*) in studies on the varietal resistance of apples to the brown rot fungus (*Sclerotinia fructigena*) that an inverse relationship exists between the proportion of wounds becoming infected and the rate of browning of injured tissue. They showed that whereas the unoxidized flavonoid compounds of the apple were relatively nontoxic to the fungus, after oxidation their toxicity, especially that of the leucoanthocyanins and catechins, increased owing to the inhibition of the pectolytic activity of the fungus.

Flavonoid compounds, themselves, have been shown to be effective in preventing fungal attack (*159, 160*), and many instances have been reported of increases in the concentration of such compounds in the tissues as a result of attack by microorganisms (*161, 162*). Such increases, although themselves significant, do not necessarily implicate the flavonoid compounds as protective agents or phytotoxins (*163*), since these changes may be effected by a disturbance in the metabolism of the plant resulting from the attack by the invading parasite. Hughes and Swain (*108*) showed that, in potatoes infected by late blight (*Phytophera infestans*), although the major phenolic compound, chlorogenic acid, increased twofold in the infected tissue, the increase in scopoletin, present only in one-hundredth of the amount of chlorogenic acid, was even more marked, being fifteen- to twentyfold. Changes of this latter order are more likely to be of significance, but it should be pointed out that until the compounds believed to be responsible for protective action are tested on the sporulation, spore germination, or growth of the parasite *in vitro*, no firm conclusions can be drawn. Cadman (*164*) has found that the leaves of many rosaceous plants contain substances tannin-like in nature which powerfully inhibit infection by plant viruses. The leaves of the raspberry, which were particularly effective, are known to contain ellagitannins, and Cadman showed that tannic acid had like properties and appeared to act by combining with the virus. Both tannic acid and the raspberry tannin combined irreversibly with some viruses, but with others the combination could be reversed by dilution or change in pH. He concluded that although phenolic compounds often seem to reach higher concentrations in virus-infected than in healthy plants (*165*), there was no evidence to suggest that such compounds protect plants from infection by viruses which have arthropod vectors or which are transmitted through the soil.

Recently Van Sumere and his co-workers (*166*) have indicated other ways in which flavonoid compounds may act to prevent infection. They have shown that the spore germination of the obligate rust parasite of wheat (*Puccina graminis*) is markedly affected by cinnamic acids (cf. *159*) and coumarins. They have shown that such compounds are widely distributed in seed coats and other dormant tissues. The fact that coumarin inhibits seed germination is of course well known (*167*), and it seems probable that other flavonoid compounds may act in this way (*168*). It is interesting to note here that many seeds from plants which do not contain leucoanthocyanins in the vegetative parts have seed coats which are rich in such substances (*141*). Such compounds might make the seed less attractive to animals because of the undesirable astringency they possess (*169*), but they may also protect the seed, if eaten, from digestion by inhibiting enzymes in the animal gut (cf. *158*).

The only other plausible function which has been ascribed to flavonoid compounds is that of soluble carbohydrate reserves (*170*). Here it was suggested that the ability of phenolic compounds to form glycosides with a number of rarer sugars, i.e., sugars not part of common metabolism, might be related to the transport of such saccharides in a nonreactive form to sites of synthesis of gums and mucilages in which they are found. In the plum, for example, phenolic compounds are associated with the exuding fruit gum and their oxidation products impart its dark color. Glycosidation, however, may serve also to protect the flavonoids themselves from attack by oxidizing enzymes (*171*, *172*) and also aid their translocation in the plant (*173*).

The further metabolism of phenolic compounds by the plant is little known. As was mentioned earlier the monomeric leucoanthocyanins may be polymerized, though whether this is a spontaneous process or not has not been investigated.

Paech and Eberhardt have shown that the leaves of noncyanic beech trees can decolorize anthocyanins extracted from the leaves of copper beech (*107*), but the enzyme responsible was not investigated. It may, however, be a β-glycosidase since Forsyth has shown that the anthocyanins of cacao are bleached by the action of such an enzyme during the fermentation process (*174*). Decolorization could then be due to the instability of the resulting anthocyanidin at the pH of the cell sap (*175*).

Hattori and his co-workers (*176*) have shown that a tissue preparation from *Cosmos sulphureus* can catalyze the transformation of chalkones into aurones. This transformation takes place spontaneously under suitable conditions of pH (*177*), but the presence of an enzyme for its catalysis, if proved, is especially interesting in view of the fact that in some plants aurones are found along with chalkones whereas other plants contain only the one class or the other.

During the discussion on biosynthesis it was pointed out that there is evidence that demethylations and dehydroxylations occur in C^{14}-labeled compounds fed to plants. The mechanism of such changes, especially the latter, is not easy to understand, but, as reported below, like changes have been observed in animals. It is possible that they are the reverse of the hydroxylations of aromatic compounds which are carried out by certain fungi (*178*).

B. IN ANIMALS

A recent Symposium was held on the Pharmacology of Plant Phenolics (*179*) and several workers reported on the action and metabolism of flavonoid compounds on the animal. It is therefore not necessary to detail recent work in full. Ingested phenolic and other aromatic compounds are metabolized in several different ways by the animal, leading to products more readily excreted or of reduced toxicity. Phenolic hydroxyl groups are usually conjugated with glucuronic acid or sulfuric acid to form glucuronides or ethereal sulfates which can be more readily got rid of via the kidney (*180*). Similarly carboxylic acid groups are often conjugated with glycine (*181*).

Hydroxylation, may also occur, usually *ortha* or *para* to an existing hydroxyl group. The resulting compound can then be either conjugated as before, or the ring may be opened by oxidation by an enzyme of the catecholase type (*85*). DeEds and his co-workers (*181*) have shown that dehydroxylation may also take place since they isolated *m*-hydroxyphenylacetic acid after feeding quercetin to rats and rabbits. The third type of action which can occur in the animal is methylation of existing hydroxyl groups. This generally takes place on the less acidic phenolic hydroxyl group of the molecule, but is not absolutely specific, since both ferulic and isoferulic acids have been found as excretion products after oral administration of caffeic acid (*182*).

The varying pharmacological actions of flavonoid and related compounds are too diverse to deal with here. Some of them are related to the interference with the normal working in the body of such phenolic substances as adrenaline, noradrenaline, and 5-hydroxytryptamine (*183*). In others beneficial effects have been claimed, as in the supposed efficacy of the flavonoids in treatment of capillary fragility (*181, 184, 185*). Certain flavonoid compounds, especially the isoflavones, are known to be effective in disturbing the estrogenic mechanism of animals (*186*), and many others are extremely poisonous to a wide range of animal life (*187*). Compounds of the latter class are, as might be expected, of rare occurrence, and it is indeed fortunate that the vast majority of flavonoids which are widespread in nature are completely innocuous since this has led to man being able to use as food material well over half of the families of higher plants.

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